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Infrared thermography of Erinaceus europaeus: Applications with hypothermia and hibernation.

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Infrared thermography of *Erinaceus europaeus:* Applications with hypothermia and hibernation

by

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Author's declaration

At no time during the registration for the degree of Research Masters in Biological Sciences has the author been registered for any other University award without prior agreement of the Doctoral College Quality Sub-Committee.

Work submitted for this research degree at the University of Plymouth has not formed part of any other degree either at the University of Plymouth or at another establishment.

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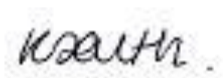
Signed: 

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Kathryn E. South

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Abstract

Erinaceus europaeus, although widely distributed across Europe, are experiencing dramatic declines throughout their UK populations. These declines place further importance on the role of rescue and rehabilitation centres in contributing to conservation of the species. Therefore reviewing and incorporating new technology and understanding into husbandry, assessment and diagnostic protocols and practices used within these rescue centres is key to maximising success rates. This study aimed to investigate the application of infrared thermography (IRT) in a rescue centre environment to hypothermia assessment as well as hibernation monitoring.

Infrared thermography (IRT) is becoming more widely available and accessible; as a non-invasive temperature assessment tool, further exploration of its uses is warranted, particularly with wildlife where minimal handling is essential to reduce stress. To assess hypothermia, a common condition of new arrivals as a result of shock, three infrared camera models (FLIR E60bx, FLIR C2 and FLIR One) were tested at Prickles and Paws Hedgehog Rescue Centre, Cubert, Cornwall. Surface eye temperature was used as a proxy for core temperature. The FLIR E60bx demonstrated increased accuracy when compared to rescue centre staff diagnosis, with a significant difference between diagnostic

categories (normal temperature, mild hypothermia and hypothermic) demonstrating misdiagnosis by centre staff. The application of IRT to skin temperature monitoring throughout hibernation demonstrated no clear pattern with weight loss that could be applied to husbandry practices, however clear patterns between spontaneous arousals, length of hibernation and weight loss were found independent of IRT skin temperature. Correlations indicate higher weight loss for longer hibernation periods but fewer spontaneous arousals, weight loss was also comparatively lower in the $\geq$700g weight category. These findings provide a basis for further research into the treatment of hypothermia in *E. europaeus* now that core temperature can be non-invasively accurately assessed and to establish the relationship between skin temperature and weight loss during hibernation.

Table of Contents

List of Tables

List of Figures

List of Appendices

List of definitions

- **Axillary temperature**: Temperature readings taken from the axilla region which is between the humerus and chest wall, which in humans corresponds to the armpit (Boden, 2005).
- **Emissivity**: The relative ability of a surface to emit and absorb radiation (Pérez de Diego *et al*., 2013), Emissivity range from 0 to 1.0.
- **Hibernacula:** Winter nests used for hibernation.
- **Hibernation**: A state of inactivity and metabolic depression to conserve energy during adverse conditions (Reeve, 1994)
- **Hyperthermia**: A body temperature greatly in excess of the normal, as occurs in fevers (Boden, 2005); within this study it was categorised as >36.3°C.
- **Hypothermia**: An abnormally low body temperature (Boden, 2005). Within this study hypothermia was split into the following categories:
    - Mild hypothermia 33.7-32.0°C
    - Hypothermic $\leq$ 31.9°C
- **Reflected apparent temperature**: A setting on some infrared cameras which compensates for radiation from the surrounding environment that is reflected by the object of focus.
- **Spontaneous arousal**: A brief awakening from hibernation, speculated to involve recovery from physiological costs accrued during metabolic depression, can involve leaving the nest, feeding/foraging or changing to a different nest (Walhovd, 1979; Reeve, 1994; Jensen, 2004). In this study the event of the individual leaving the nest was defined as 'spontaneous arousal'.

- **Synanthropic species:** A species that is closely associated, lives in, near or around human environments (Pfäffle, 2010).

Abbreviations

IRT – Infrared thermography

RAT – Reflected apparent temperature

TMT – Tympanic membrane temperature

Chapter 1: Introduction

1.1 *Erinaceus europaeus*

Erinaceus europaeus, of the family Erinaceidae, is commonly referred to as the European hedgehog and is Britain's only spiny mammal (Gould and Partridge, 2013). Found across Europe (Figure 1), this nocturnal species is considered non-territorial (Reeve, 1994). The species is highly adaptable and can survive in a variety of climates, in rural or urban locations, commonly in areas with deciduous trees and bushes (Sykes and Durrant, 1995; Thamm *et al.,* 2009; Dowding *et al.,* 2010). In Denmark, the two main habitats within their home ranges were deciduous forest and arable land, whilst the most used habitats were deciduous forest and grassland (Riber, 2006). *Erinaceus europaeus* plays an important ecological role as tertiary consumers within the food chain with a flexible diet which includes large numbers of invertebrates, small vertebrates and carrion (Molony *et al.* 2006; Naem *et al.,* 2014); approximately 80% of active time is spent foraging, regardless of sex (Wroot, 1984).

Erinaceus europaeus is currently classified by the International Union for Conservation of Nature (IUCN, 2017) as of Least Concern, however small-scale studies demonstrate a significant decline in the British population (Battersby, 2005; Macdonald and Baker, 2006; Gaglio *et al.,* 2010) and a recent report by the Mammal Society (Matthews *et al.,* 2018) lists them as vulnerable on the regional red list assessments.

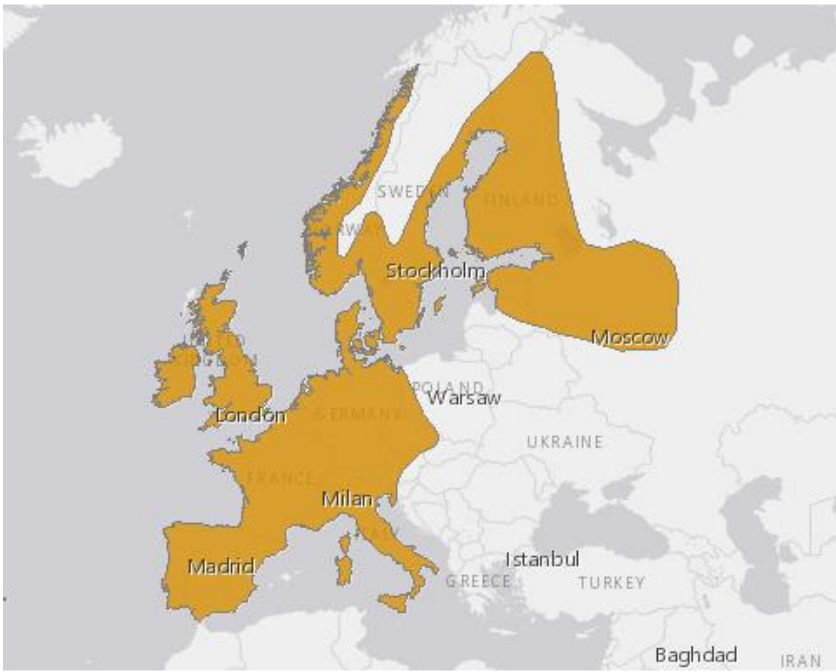


Figure 1: Erinaceus europaeus distribution in Europe. The species has been introduced to New Zealand (not shown here) (IUCN, 2017).

1.2 Wildlife rehabilitation

Wildlife rehabilitation is defined as "the managed process whereby a displaced, sick, injured or orphaned wild animal regains the health and skills it requires to function normally and live self-sufficiently" (Molony *et al.,* 2006, p. 530). The RSPCA reported 17,202 wild animals admitted to their centres across the UK in 2016, their admissions consisted of 76% birds and 23% mammals, with *E. europaeus* the most commonly admitted species in 2012 (RSPCA, 2017). No breakdown of species admitted is available for 2016 or 2017. There are a number of wildlife rescue centres across the UK which range from admitting single species to all wildlife (Stocker, 2005). Similarly to the RSPCA figures, Molony *et al.* (2006) report *E. europaeus* as the most common mammal species admitted to rescue centres across the UK. It was estimated in 2006 by Stocker (Tiggywinkles Wildlife Hospital), that between 30,000 and 40,000 wildlife casualties are admitted to wildlife hospitals and rescue centres each year (Molony *et al.,* 2006).

1.3 *Erinaceus europaeus* rehabilitation

Figures collected from 12 rescue centres/wildlife hospitals across the UK demonstrate an increase in numbers of *E. europaeus* admitted from 2015 to 2016, with two centres admitting over 1000 casualties in 2016 (Wild Hedgehog Rehabilitators 2017, Pers. comm. 27 February; Appendix 1). The British Hedgehog Preservation Society (2017) has 737 carers and rescue centres listed in the UK (F. Vass 2017, Pers. comm., 2 March), however this is only those who have chosen to list with the society. There is no centralised database of those people involved in wildlife rehabilitation, their qualifications or a standard code of practice and conduct (Mullineaux, 2014). Morris (2014) believes there has been a significant increase in the number of people caring for sick, injured or orphaned *E. europaeus* over the last 30 years, in part due to the awareness and publicity given to their decline and the threats they face. There are no licensing restrictions associated with keeping wildlife, specifically *E. europaeus,* in captivity in the UK, however wildlife law and legislation applies whether a wild animal is in the wild or in captivity. Wildlife is also protected under animal welfare legislation, preventing cruelty and imposing restrictions upon capture and treatment: Animal Welfare Act 2006, Protection of Badgers Act 1992, Deer Act 1991, Wildlife and Countryside Act 1981, Abandonment of Animals Act 1960 and the Veterinary Surgeons Act 1966 (Belle, 2000; Mullineaux, 2014) priority species under the former UK Biodiversity Action Plan (Wilson and Wembridge, 2018).

Molony *et al.* (2007) report the most important predictive factors in re-release of wildlife admitted to rescue or rehabilitation centres were not age or mass but the severity of the symptoms of illness or injury. Williams (2010) reports that it is common for wildlife casualties, including *E. europaeus,* to die of shock within 24 hours of admission; a key symptom of this is hypothermia (Stocker, 2005). This highlights the importance of triage based on clinical assessment as soon as a casualty is admitted and evidence based rehabilitation protocols to optimise survival rates. Bullen (2013) lists hypothermia, alongside dehydration and myiasis (flystrike), as the three main life-threatening conditions for *E. europaeus* casualties, therefore appropriate and accurate diagnosis and treatment is essential.

The success rate of post-rehabilitation *E. europaeus* wild release is largely attributed to their flexible diet allowing re-release at novel locations as they can readily adapt to their new environment (Molony *et al.,* 2006). Direct translocation, as opposed to release post rehabilitation, had a negative effect on survival of *E. europaeus* whilst only a short period in captivity prior to release improved the chance of survival by allowing the build-up of fat reserves (Molony *et al.,* 2006). These beneficial effects were also evident for individuals retained in captivity for a period of rehabilitation following illness or injury prior to release (Molony *et al.,* 2006). A large number of *E. europaeus* will remain in captivity over winter months due to unfavourable environmental conditions, such as low or close to freezing temperatures or heavy rainfall reducing dry nest site availability, delaying release (Bullen, 2010). Releasing in these unfavourable conditions could result in weight loss and therefore increase the risk of hibernation by not having sufficient fat reserves. This is especially important to consider as Millineaux and Keeble (2016) refer to hibernation as the single greatest mortality factor for the species with up to 70% of young *E. europaeus* dying in their first winter.

With such a reported decline across the UK and many admitted to rescue centres and wildlife hospitals, improving rehabilitation protocols and survival rates has the potential to advance both animal welfare and conservation; particularly as Molony *et al.* (2007) reported that less than half of all wildlife casualties are subsequently released back into the wild.

1.4 *Erinaceus europaeus* decline

Harris *et al.* (1995) estimated a population of 1.55 million *E. europaeus* across England, Scotland and Wales, however, the most recent estimate is 522,000, a 66% reduction (Matthews *et al.,* 2018).Both Matthews *et al.* (2018) and Wilson and Wembridge (2018) analysed historic and ongoing studies, identifying a decline in rural populations and although they do not appear as widespread as previously recorded, those places with urban populations are seeing a slight increase in numbers in the last couple of years, the reasons for this change remain unclear at present. The studies analysed to produce this conclusion have markedly different methodologies, different timeframes, recording techniques and sample area, that makes comparisons challenging. Wilson and Wembridge (2018) also identify that within these long-term studies there have been changes to the survey methods which cast further uncertainty on the trends identified. The key observation however is that all studies analysed by Wilson and Wembridge (2018) show a decline from Morris' original 1989 study, with no studies deemed to be producing results reflective of actual population size due to *E. europaeus'* nocturnal and secretive nature making accurate monitoring difficult (Wilson and Wembridge 2018).

The clear decline in *E. europaeus* and the difficulty in carrying out a population census places further importance upon rehabilitation centres and their success in returning individuals to the wild. The causes for decline are primarily anthropogenic and include: garden and pet injuries, habitat loss (conversion), fragmentation and disturbance as well as road traffic accidents (Sykes and Durrant, 1995; Reeve and Huijser, 1999; Huijser and Bergers, 2000; Rondinini and Doncaster, 2002; Thamm *et al.* 2009). Habitat destruction and fragmentation is a major factor, with modern agricultural practices leading to the removal of hedgerows in order to create larger, more efficient fields to cultivate and the reduction in field margins, in addition to the construction of roads and housing estates (Sykes and Durrant, 1995; Wilson and Wembridge, 2018). Within agriculture and urban land, toxic and poisonous chemicals are commonly applied, for example metaldehydge in slug pellets potentially poisoning *E. europaeus* as well as reducing their prey (Sykes and Durrant, 1995; Huijser and Bergers, 2000). Sykes and Durrant (1995) consider mechanization (motor vehicles, strimmers and machinery) the most important cause of decline. In the Netherlands roads and traffic were likely to reduce *E. europaeus* population density by 30%, which may affect the survival probability of local populations (Huijser and Bergers, 2000). As a synanthropic species many anthropogenic factors appear to contribute to their decline, however biotic and abiotic factors must also be considered, including parasitic burdening (Pfäffle, 2010).

A considerable number of mortalities are likely due to natural causes, including parasitic burdening (Gaglio *et al.,* 2010). High parasite burdens have the potential to reduce reproductive success and survival in wild mammals, often through their effects upon body condition (Irvine, 2006). *Erinaceus europaeus* hosts a range of endoparasites and ectoparasites (Appendix 2; Naem *et al.,* 2014). Many endoparasites are obtained through their diet, including the nematodes *Capillaria* spp. and *Crenosoma striatum* which are transmitted through intermediate hosts (Haigh et al., 2014). *Crenosoma striatum* are commonly transmitted via molluscs (land snails and slugs), whilst molluscs and earthworms host *Capillaria aerophila*, but only earthworms host the other two *Capillaria* spp. (*Capillaria erinacei* and *Capillaria ovoreticulata*) (Gould and Partridge, 2013). Majeed *et al.* (1989) add that *Capillaria* spp. can also be transmitted directly without an intermediate host; this however is not reported in other literature. High levels of parasitic burdening can indicate reduced health and be a reason for admittance to rescue centres (Reeve, 1994; Millineaux and Keeble, 2016).

Diet composition varies on a seasonal basis, with individuals tending to swap between four main prey types which provide the bulk of their energy: 'carabid beetles, earthworms, Lepidoptera larvae, and tipulid larvae' (Wroot, 1984, pp.2). This perhaps indicates that parasitic infection or burdening may vary throughout the year as they demonstrated a tendency to concentrate on one prey type at a time and switch between them on a seasonal basis (Wroot, 1984). Anecdotal evidence from rescue centres suggests parasitic infection, specifically by *C. striatum,* is an important cause of illness in *E. europaeus* (Gaglio *et al.,* 2010). In the context of rehabilitation, this has implications for diagnosis; detection will be affected by the length of time the infected mollusc was ingested before admittance. *Crenosoma striatum* larvae can take up to 21 days following ingestion to be present in faeces (Pfäffle, 2010). Ensuring rescue and rehabilitation protocols are effective and targeted is key to the conservation of the declining population. Parasitic burdening may become an even more important factor in their decline in the future as climate change is likely to impact on host-parasite population dynamics (Hakalahti *et al.,* 2006; Pfäffle, 2010), as well as habitat fragmentation affecting their home range (Sykes and Durrant, 1995) and therefore diet. Rescue and rehabilitation protocols therefore require constant review and modification to ensure that they are reflective of the likely condition or parasite burden of the individuals admitted.

Home range is a term used to describe the area in which an animal normally or commonly travels and occupies in search of food, mates or resources (Reeve, 1994). Home ranges are not fixed and can both overlap and change in response to environmental conditions (Reeve, 1994). Estimates of range size for *E. europaeus* do vary; a review by Reeve (1994) estimates areas of between 5.5ha and 102.5ha. Many studies have examined home ranges in *E. europaeus* with the general consensus that the home range of males is larger than that of females (Reeve, 1994; Huijser and Bergers, 2000; Riber, 2006; Dowding *et al.,* 2010), with estimates of between 32 and 47ha for males, and of 10 to 20ha for females (Huijser and Bergers, 2000). The home range size is reported to change throughout the year particularly for males, with a larger range during spring when searching for mates (Dowding *et al.*, 2010). Some rescue centres report a statistically significant male biased sex ratio of 1.2:1 (m:f) in total admissions and note that the stronger bias occurred early in spring (Reeve and Huijser, 1999) which is when their range is at its greatest (Dowding *et al.,* 2010). This male bias could be a feature of their larger home range with a greater chance of human contact and, for sick individuals, of being picked up by a member of the public and admitted to a rescue centre. A larger home range could allow greater contact with intermediate parasite hosts, resulting in greater risk of endoparasitic infection, again linking to their admittance to rescue centres. This is supported by Haigh *et al.* (2014) who reported a significantly higher loading of the parasite *C. striatum* in male *E. europaeus* compared to females.

1.5 *Erinaceus* europaeus rehabilitation protocols

Assessment and monitoring protocols for *E. europaeus* at rescue centres vary between individual centres, although there are standard guidelines available from Vale Wildlife Hospital (Gould and Partridge, 2013). When admitted to a rescue centre an initial assessment is carried out to assess the requirements for first aid and stabilisation, to assess the viability of rehabilitation, to reach a diagnosis and institute initial treatment (Gould and Partridge, 2013; Bullen, 2010). Initial treatment will usually constitute fluid therapy to treat shock and dehydration, warmth for hypothermia, stabilisation of fractures or wounds and analgesia for pain if appropriate (Gould and Partridge, 2013; Bullen, 2010). Once the casualty is stabilised (warmth and rehydration) wounds can be cleaned and further assessments carried out, such as faecal sampling (Gould and Partridge, 2013). Despite it being considered standard protocol for newly arrived *E. europaeus* to be treated for shock and hypothermia on arrival at rescue centres, there is no clear way described of assessing the degree of hypothermia by either Gould or Partridge (2013) or Millineaux and Keeble (2016). Due to *E. europaeus* ability to tightly curl a rectal temperature is not obtainable for most casualties and would be very stressful (Robinson and Routh, 1999), therefore diagnosis is largely based on touch and behavioural cues which are more subjective and does not allow an accurate degree/level of hypothermia can be ascertained. The result of this is that the treatment for hypothermia is non-specific and is limited to fluid therapy and gradual rewarming through a heat pad, incubator, chick brooder or heat lamp (Millineaux and Keeble, 2016) as no initial core temperature is obtainable to instigate a rewarming protocol similar to those applied in medicine.

Once stabilised and a treatment plan put in place Gould and Partridge (2013) recommend daily weighing of individuals, this ensures accurate dosage of any medication administered and provides information on if the individual is eating enough or needs supportive hand feeds. Once an individual has completed all treatments and is fit, healthy and an optimum weight release is arranged as soon as possible to reduce the time and associated stress of being in captivity (Gould and Partridge, 2013). During the winter, at Prickles and Paws Hedgehog Rescue, when unfavourable conditions prevent release the frequency of weighing is reduced for those individuals which are healthy and of an optimum weight to reduce disturbance and allow hibernation. Bullen (2010) does not provide guidance on frequency of weighing during hibernation, however the centre weigh on a six to eight weekly basis to check that bedding remains dry and that individuals have not fallen below the 450g, the recommended limit below which survival from hibernation is reduced (Bullen, 2010). Bullen (2010) also notes that it is not essential *E. europaeus* to hibernate, however the effects of not hibernating on their seasonal cycle and hormone levels has not been studied.

1.3 Infrared thermography (IRT)

With large numbers of *E. europaeus* admitted to rescue centres across the UK there is cause to review treatment and husbandry protocols of the species. As infrared technology reduces in cost and becomes more widely available its range of applications has increased (McCafferty, 2013), and is being further explored. Infrared thermography (IRT) is a non-invasive temperature measurement tool which uses the ability of all objects to emit infrared radiation, as a function of their temperature, producing a pictorial representation (Figure

2) of their temperature (Stokes *et al.,* 2012). The radiation emitted by an object is proportional to its surface temperature (McCafferty, 2007).

There are no published studies monitoring *E. europaeus* temperature using IRT although Haigh *et al.* (2012), tried to detect the species in the wild. Using a Testo 880 infrared camera in arable, garden and pasture environments they failed to detect radio tagged *E. europaeus* at distances greater than 1m. In contrast, Reeve (2016, Pers. comm., 10 December) successfully detected *E. europaeus* within Regent's Park, London, using a FLIR E60 infrared camera. The cameras were used to locate individuals for marking and then radio/GPS tags were attached to allow study of movement, nest sites, behaviour and habitat utilisation (Reeve, 2016, Pers. comm., 10 December). The success of the cameras in detecting *E. europaeus* was not quantified within the study, but the conclusion of the authors was that IRT was very effective in detecting *E. europaeus* at up to 60m away in open habitats.

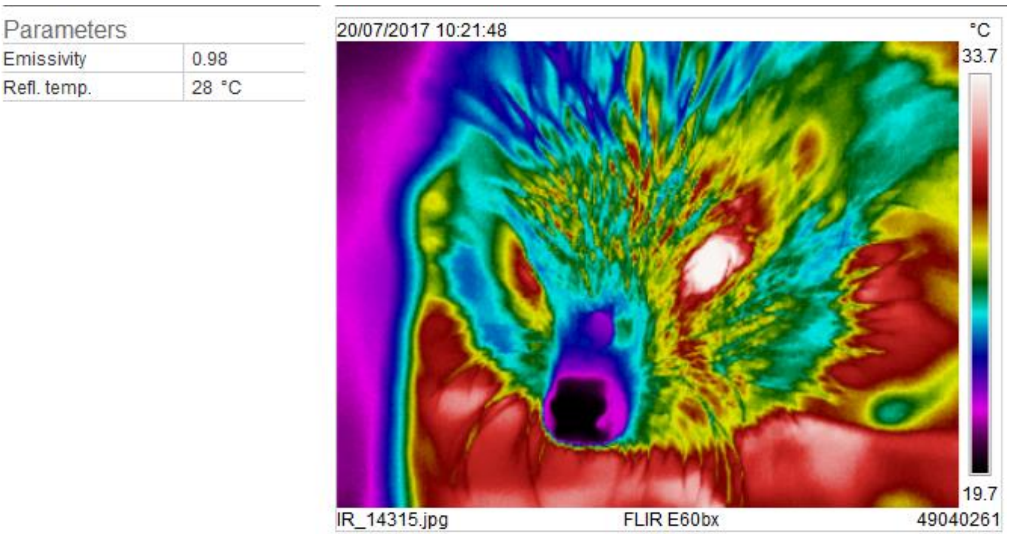


Figure 2: Infrared image of an *E. europaeus* taken using a FLIR E60bx (South, 2017).

It has been well documented that surface body temperature is directly linked to underlying circulation. Radiative heat-loss can be used successfully as an early indicator of fever (Schaefer *et al.,* 2004; Vadlejch *et al.,* 2010; Pérez de Diego *et al.,* 2013). Fever detection is a common application which extends to humans as a screening tool at border crossings and airports in periods of disease outbreak (Chan *et al.,* 2013). Conversely hypothermia detection, in any species appears relatively unexplored. IRT however is gaining in popularity as a diagnostic tool for evaluating human and animal health as it can reduce subject stress due to its non-invasive nature (Soerensen and Pedersen, 2015).

IRT has been widely used in the veterinary field to aid in assessment and diagnosis, including the early detection of mastitis in cows (Colak *et al.,* 2008), rabies virus in racoons (Dunbar and MacCarthy, 2006) and foot-and-mouth disease virus in cattle (Rainwater-Lovett *et al.,* 2009). Pérez de Diego *et al.* (2013) reported significant changes in facial temperature of sheep infected with bluetongue virus. The study reported that fever was the earliest clinical sign displayed and that IRT was an effective screening tool for this. Despite considerable success in diagnosing raised body temperatures IRT does not appear to have been widely utilised for hypothermia assessment. Thermography of species which hibernate and the process of hibernation have been examined (Pajunen, 1984; Phillips and Heath, 2001; Phillips and Heath, 2004), but not in *E. europaeus*, where there is a high mortality reported in juveniles (Millineaux and Keeble, 2016).

IRT protocols and methodology vary with species and anatomical area examined. Stress can affect core and skin temperature (Soerensen and Pedersen, 2015). The handling and confinement of any casualties can cause significant stress. (Stocker, 2005). The non-invasive nature of IRT is a key to the reduction or elimination of potentially life threatening shock. Thermal infrared cameras currently available on the market vary in cost, size and capability. Different cameras and models must therefore be tested for each application as well as species. Many studies have validated temperature values extracted from infrared images by collecting simultaneous temperature data through either rectal or implanted temperature data loggers (Rainwater-Lovett *et al.,* 2009; Pérez de Diego *et al.,* 2013). Over a three year period studying *E. europaeus* Fowler and Racey (1990) used intra-abdominal implanted temperature sensitive radio transmitters, they report a July maximum of 35.9±0.2°C, and September minimum of 34.7±0.9°C. An older study of only male *E. europaeus* reported a range of 34.7 - 36.2°C where temperature sensors were inserted and allowed to move freely within the peritoneal cavity (Fowler and Racey, 1987).

Another key factor, and perhaps limitation to IRT, is that environmental conditions can influence the results (McCafferty, 2007) and therefore a cameras ability to standardise these (which will change depending on the model of camera) must be considered in protocols. Vadlejch *et al.* (2010) specify recording air temperature, ambient temperature, relative air humidity and velocity (microclimate conditions), whilst Dunbar *et al.* (2006) record only ambient temperature and humidity (two factors which can be set on a FLIR E series camera). It is unclear whether these factors most influence IRT, or whether they are simply the easiest to record. This raises the question of the importance, or effect, of specific environmental conditions and parameters on IRT results; perhaps indicating a lack of understanding surrounding the nature of IRT (Stewart *et al.,* 2007; Vadlejch *et al.,* 2010; Stokes *et al.,* 2012).. Similarly few studies appear to control for, or account for, reflected apparent temperature (RAT) nor the method used to calculate, particularly in the field of animal health monitoring. It is recognised that incorrect RAT, alongside emissivity values, can affect accuracy of temperature data extracted from infrared images (Lathlean *et al.,* 2012). The Infrared Training Centre (2012) provides an easy method ('the foil method') for calculating RAT, although this does not yet appear in any available published literature within this or any related field. As IRT is applied in new fields, a lack of adequate knowledge (Meola and Carlomagno, 2004) has resulted in some of the more complex features of the higher range cameras being overlooked or incorrectly set as very few papers refer to RAT or its calculation. For this technology to be implemented into a rescue setting there needs to be protocols that are easy to follow.

1.4 Project aims

This project aims to inform the further development of diagnostic and rehabilitation protocols of *E. europaeus* within rescue centres. The perceived rapid decline of the species within the UK places an ever growing responsibility on rescue centres to increase rehabilitation success rates, contributing to conservation through those which individuals which are released back into the wild. Application of newly available technology, such as IRT, has the potential to increase rehabilitation success rates with evidence based protocols and understanding. Specifically, this project investigates the use of IRT as a non-invasive diagnostic aid for hypothermia and a temperature monitoring tool for hibernation which can be combined with observations of spontaneous arousal to inform husbandry. Diagnosing hypothermia in a non-invasive manor using IRT would allow the most appropriate treatment to be given, stress to the individual to be reduced through reduced handling, improved welfare and survival rate. The monitoring of hibernation using IRT has the potential to reduce handling, allowing most natural hibernation, improving welfare and reducing the risk of death by non-invasive monitoring combined with spontaneous arousal patterns used to inform handling and weighing practices.

Chapter 2: Hypothermia

2.1 Hypothermia and shock

Shock is defined, clinically, as an abrupt fall in blood pressure/ acute hypotension (Boden, 2005). The key symptoms of shock are subnormal temperature but no shivering, weakness, pale and cold mucous membranes, a weak and rapid pulse, shallow breathing at an increased rate and cold extremities (Boden, 2005). The causes of shock include: trauma, haemorrhage, surgical operations, burns / scalds, pain and fright (Millineaux and Keeble 2016). It is commonly accepted that all wildlife casualties will be suffering from some degree of shock when admitted to a rescue centre due to stress and fright of being handled and transported regardless of injuries or disease (Bullen, 2010; Gould and Partridge, 2013; Millineaux and Keeble 2016).

The recommended treatment for shock in wildlife, specifically *E. europaeus,* is fluid therapy and slow rewarming, as well as treatment of any haemorrhage or active bleeding (Bullen, 2010; Varga *et al.,* 2012; Gould and Partridge, 2013; Millineaux and Keeble 2016); however there is limited guidance on a rate of rewarming besides 'slow'. Varga *et al.,* (2012) advises providing a rewarming environment such as an incubator between 20 and 25°C whilst Bullen (2010) recommends placement on a covered hot water bottle. Treatment is non-specific and subjective, similar to diagnosis.

Hypothermia, as one of the key symptoms of shock, is defined as an abnormally low body temperature (Boden, 2005), there are no degrees or levels of hypothermia defined to guide rewarming protocols. The causes, symptoms and treatment advised in veterinary practice are outlined in Table 1, not all are transferable to assessment in *E. europaeus*, others such as assessment of mucous membranes and capillary refill time may depend upon the severity of hypothermia and presentation of the casualty.

Causes of hypothermia	**Symptoms**	**Treatment**
- Shock - Trauma - Haemorrhage - Fright/stress - Exposure to elements - Starvation - Weakness - Immersion - Anaesthesia	- Unsteady / wobbly / jerky movement (can appear drunk) - Cold to the touch - Cold extremities - Semi-conscious - Stomach feels cold to the touch - Gums cold to the touch - Low blood pressure / low capillary refill time - Shallow breathing - Shivering - Peripheral pulses decrease	- Placement in a quiet and dark environment - Fluid therapy - Heat (can be in the form of a heat pad, chick brooder or incubator - If at a veterinary practice oxygen may be provided - Sternal recumbency positioning to aid breathing

Table 1: Causes, symptoms and treatment of hypothermia (Boden, 2005, Bullen, 2010; Varga *et al.,* 2010; Millineaux and Keeble, 2016).

In wildlife casualties often the cause and duration of hypothermia is not known and therefore neither are the effects on the body and organ systems. As a hibernating species some of the risks of hypothermia may be reduced for *E. europaeus.* De Vrij and Henning (2015) evidenced that hibernating species have a key adaptation to their coagulation systems where they are able to fully reverse the state of thrombocytopenia (low blood platelet count). Reversible adherence of platelets to vessels by means of margination depletes circulating platelets by up to 96% in hamsters, preventing blood clots from forming; however De Vrij and Henning (2015) concluded that it is unclear whether this is induced by the mechanism causing hibernation or indirectly by the low body temperature (hypothermia). Medical research conducted by Poucke *et al.,* (2014) supports the latter, indicating increased margination in response to hypothermia. If this is true in *E. europaeus* this may have implications for the treatment of hypothermia in the species and the decision making process with such treatments as the risk of blood clots may be greatly reduced. Other known effects of hypothermia on the mammalian body systems, include reduced immune function (Bouma *et al.,* 2010). Hypothermia has been shown to suppress and delay immune responses, specifically reduced cytokine release and production, antibody production and lymphocyte proliferation, greatly increasing the risk of infection (Bouma *et al.,* 2010).

Hypothermia is commonly used in medical science for its neuroprotective effects (Thoresen *et al.,* 1995; Suehiro *et al.,* 2004) however in the case of wildlife casualties rewarming of hypothermic casualties may exacerbate problems which were not apparent due to the hypothermia masking or reducing them. It may be the case that many mildly hypothermic cases are not identifiable using the symptoms outlined in Table 1, but either improve through nonspecific shock/hypothermia treatment of fluid therapy and a warm, quiet environment or are detected after further deterioration. Presently there is no evidence to confirm either of these scenarios as in most cases a core temperature is not known. Both shock and hypothermia can be fatal (Boden, 2005). A history is rarely provided to the rescue centre detailing the cause of hypothermia, for example trapped in a drain/pond or trauma causing internal bleeding. In the cases where there are no external signs, such as broken prickles indicating trauma, establishing a temperature is a diagnostic tool to indicate the most appropriate and targeted treatment which would cover all potential causes.

Symptoms of hyperthermia, a body temperature greatly in excess of normal (Boden, 2005) differ from those of shock and hypothermia. These include panting, laying stretched out, hot to the touch, seizures (Millineaux and Jones, 2007; Aldridge and O'Dwyer, 2013). The causes of hyperthermia can include heat stroke, exercise, seizures and incorrect use of heat pads or heat sources (Millineaux and Jones, 2007).

2.1.1 Health and temperature assessment

On arrival at rescue centres *E. europaeus* casualties go through a triage / assessment. Obtaining a rectal temperature without the use of anaesthetic, a facility most rescue centres do not have, is very difficult and a procedure not appropriate for new arrivals suffering from shock, especially as anaesthesia disrupts the body's ability to thermoregulate (Aspinall, 2011; Wells, 2016). Stress is commonly caused by capture, management and manipulation (handling) of animals during clinical examination (Pérez de Diego *et al.,* 2013). There are currently no non-invasive technologies commonly used in a rescue/wildlife hospital setting to aid in assessment and diagnosis, although remote wildlife cameras/ camera traps can be used in monitoring, particularly post release at nest boxes or feeding stations.

Establishing a core temperature is a key aspect of health assessment (Aspinall, 2011). Rectal temperature, obtained from the rectum mucosa, has long been considered the 'gold standard' for core or body temperature measurement in veterinary practice (Sousa *et al.,* 2011) and can take 30 - 60 seconds (Boden, 2005). In *E. europaeus* a rectal temperature cannot easily be obtained without the use of anaesthetic due to their ability to curl into a tight ball (Robinson and Routh, 1999).

Rectal temperature remains the traditional measure for core temperature, however due to the stress associated with obtaining a rectal temperature as well as a risk of cross-contamination, other temperature methods are being explored (Sousa *et al.,* 2011). Tympanic membrane temperature (TMT) taken using an infrared auricular thermometer reduces the time needed to obtain a temperature reading. Michaud (1996) reports that as little as 2 seconds is required, and is considered less stressful due to its less invasive nature, resulting in more compliant patients, particularly in cats and dogs (Sousa *et al.,* 2011; Hall and Carter, 2017). The thermometer utilises pyroelectric sensors to detect temperature of the tympanic membrane, which theoretically provides an accurate measure of core body temperature (Brinnel and Cabanac, 1989; Sousa *et al.,* 2011). Michaud (1996) reported a significant positive correlation of TMT and rectal temperatures in cats ($r = 0.995$, $p<0.001$). More recent studies have examined the use of TMT with dogs; Hall and Carter (2017) concluded that TMT measured consistently below rectal temperature. Application of a 0.4°C correction factor minimised the difference and TMT could be used to monitor body temperature in exercising dogs where a rectal temperature was not obtainable. In human studies TMT diverges below rectal temperature as body temperature increases in exercise induced hyperthermia (Huggins *et al.,* 2012) but this was not observed in dogs (Hall and Carter, 2017).

Axillary temperature is considered easily obtained in dogs, however the readings were lower than rectal temperatures (median difference of 0.6°C), with weight, coat, body condition score and breed size significantly associated with the difference between rectal and axillary temperature (Lamb and McBrearty, 2013). Dogs were more tolerant of axillary measurements than they were TMT (Lamb and McBrearty, 2013). In *E. europaeus* obtaining an axillary reading is realistic with the individual curling around the thermometer, however a TMT reading is less achievable due to the tympanic membrane being inaccessible when tightly curled unless anaesthetic is used. Infrared thermography has many of the same benefits of TMT but is even less invasive which reduces the risk of spreading infection since touching the subject is not necessary (Soerensen and Pedersen, 2015).

2.1.2 IRT specific eye methodology and application

Although general IRT methodology and application of camera settings are still being developed and refined (refer to section 1.3), specific methodology relating to the anatomical area of the eye surface has been considered in some depth. The emissivity of the human eye has been estimated at 0.98 (Girardin *et al.,* 1999), this figure has also been applied to sheep eyes by Pérez de Diego *et al.* (2013), however none of Schaefer *et al.* (2007), Rainwater-Lovett *et al.* (2009), Vadlejch *et al.* (2010), McGreevy *et al.* (2012) and Schaefer *et al.* (2003) list in their methods the emissivity values used for infrared imaging of the eye surface in their study species. Ludwig *et al.* (2007) supposed the emissivity of a rabbit's eye surface to be the same as water, but do not state 1.0, which is the accepted value for water. The implication of this is that the relationships described in these studies may not be accurate or comparable and future studies must aim to standardise and apply these variables reporting clearly the settings and emissivity used.

Pérez de Diego *et al.* (2013) report that infrared surface eye temperature was positively correlated to rectal temperature ($r = 0.504$, $p < 0.05$, $n = 18$) in sheep. They also note that the highest correlation between rectal and eye temperature was observed when temperatures were above normal physiological levels. IRT was able to discriminate between febrile (hyperthermic) and non-febrile sheep with sensitivity of 85% and specificity of 97%. Similar levels of sensitivity (74.6%) and specificity (92.3%) have been found in detecting febrile ponies (Johnson *et al.,* 2011). Pérez de Diego *et al.* (2013) employed the same method as Schaefer *et al.* (2007), extracting the maximal eye temperature from infrared images, obtained by tracing a circle over the orbital area including the eyeball and approximately 1cm of surrounding skin of the eye socket, whilst other studies specify only the maximal eye temperature (Rainwater-Lovett *et al.,* 2009; Johnson *et al.,* 2011). None of the above studies have considered or discussed the effects of blinking on eye surface temperature, however Tan *et al.* (2009) report that eye surface temperature is correlated with body surface temperature and tear film stability after blinking. Multiple studies on a range of species conclude that IRT has the potential to be a successful temperature and disease screening tool (Schaefer *et al.,* 2004; Schaefer *et al.,* 2007; Vadlejch *et al.,* 2010; Johnson *et al.,* 2011; Pérez de Diego *et al.,* 2013). Only Vadlejch *et al.* (2010) looked at decreased body temperatures, in rabbits, as opposed to raised or febrile temperatures. There is certainly room for investigation into the application of IRT for hypothermia detection across a range of species.

2.2 Aims

This aspect of the study aims to investigate the application of three infrared cameras (FLIR E60bx, FLIR C2 and FLIR ONE) for diagnosing hypothermia in *E. europaeus* at rescue centres..

The purpose of which will be to inform and potentially add a new tool to husbandry practice and protocols to increase survival and re-release rates, contributing to conservation by maintaining/boosting numbers of this declining species. The study aims to establish eye surface temperature as a proxy for core temperature within the species and apply this to diagnosing hypothermia non-invasively in newly admitted *E. europaeus,* comparing these results to staff diagnosis based on observation of symptoms and behaviour. A separate thermal calibration trial will aim to establish differences between the three cameras.

2.3 Methodology

The study was conducted between 2015 and 2017 on *E. europaeus* admitted to, and undergoing rehabilitation at, Prickles and Paws Hedgehog Rescue Centre in Cornwall, UK. The rescue centre protocols were incorporated to exploit opportunities for data collection while individuals were already being handled so no additional handling or stress was caused which impacted upon their rehabilitation. When an eye image could not be taken during handling the individuals could be observed once they were placed during their hutch, this could be done in complete darkness which reduced their stress resulting in them uncurling.

2.3.1 IRT Methodology

All infrared images were recorded using the FLIR E60bx, FLIR C2 and FLIR ONE (listed in descending cost order with the assumption that this will affect capability of the cameras, respective camera data sheets Appendix 3, 4 and 5). Ambient temperature and humidity were recorded, using a mini digital thermometer and humidity meter, and values set accordingly on the FLIR E60bx. Reflected apparent temperature (RAT) was calculated and set, on the FLIR E60bx and FLIR C2 cameras, using the foil method (Infrared Training Centre, 2012). The crumpled foil, placed in the same plane as the target, acts as a diffuse infrared reflector, reflecting background radiation. Using an emissivity of 1.00 the average temperature is taken and set as the RAT (Infrared Training Centre, 2012). This method is simple and easily replicated, however not used or reviewed in zoological or veterinary literature, it appears to be a method/principle more utilised in the building industry (Dactu *et al.,* 2005).

Emissivity on the FLIR E60bx and FLIR C2 cameras was set at 0.98, the value used for the surface of a sheep's eye suggesting suitability for *E. europaeus* and other mammalian eyes (Girardin *et al.,* 1999; Pérez de Diego *et al.,* 2013).. Images were taken between 0.2 and 0.4m from the subject as determined by focus and clarity of the eye, this distance was set on the camera as <1m on the FLIR E60bx and FLIR C2. The FLIR C2 screen displays a circle of the object being measured and the object or study area being analysed needs to fill the circle entirely to obtain an accurate temperature reading at the time of imaging. The FLIR ONE camera, and iPhone app to operate the FLIR ONE camera, did not present the same circle as the FLIR C2 so images had to be taken closer to ensure enough pixels overlaid the target area to give an accurate temperature. Thus, FLIR ONE images were taken 0.2m from the subject despite the camera model having the same IR sensor as the FLIR C2 (Appendix 4 and 5). The emissivity for the FLIR ONE camera was set at the pre-set value of 0.95, once downloaded, images can be manipulated in the FLIR Tools software (FLIR: version 5.6.16078.1002) to correct the emissivity to 0.98 and set the reflected apparent temperature, recorded independently at the time of imaging.

For all aspects of the study multiple consecutive images were taken. One image was then selected for analysis based on the criteria of: focus, area visible, amount of skin visible through the prickles or openness of the eye. Eye surface and skin temperatures were extracted from selected infrared images using FLIR Tools software.

2.3.2 Thermal calibration

A pilot study was carried out to assess possible variation between the three different model cameras and establish apparent differences within temperature readings. Images were taken of a piece of electrical tape (Scotch, Professional grade vinyl electrical tape), with a known emissivity of 0.95, placed on the outside of a tin can containing water at 13 known temperatures ranging between 10 - 47°C (measured to two decimal places using a digital thermometer). Infrared readings of the tape and the water temperatures should be similar due to the high emissivity of the tin and low emissivity of the tape conducting heat from the water (FLIR, 2015). An emissivity of 0.95 was set on all three cameras; ambient temperature was corrected during analysis on FLIR Tools. To test consistency among cameras of the same model the trial was repeated using two FLIR ONE cameras (10 known temperatures between 10.9°C - 47°C). The FLIR ONE used in the first trial was termed ‘FLIR ONE Original’ and a second FLIR ONE camera was termed ‘FLIR ONE New’. As the cheapest camera (Appendix 5) in this study, and therefore most suited to the rescue centre setting, the aim of this subsequent trial was to establish that the results are representative of the camera type and not the result of calibration differences or discrepancies in sensitivity and temperature readings.

2.3.3 *Erinaceus europaeus* baseline temperature assessment

To establish ‘normal’ variation in eye temperature, which was used as a proxy for core temperature, the IRT (FLIR E60bx) eye surface temperatures of healthy individuals (all over 300g and therefore classed as juvenile or adult) were compared to previous studies reported core temperature ranges for the species (Fowler and Racey, 1987; Fowler and Racey, 1990). Five healthy males and five healthy females were imaged using the FLIR E60bx between 22/01/17 and 24/01/17. All individuals were sampled on all three days in the same environment. Over the three days ambient temperature varied between 15.9°C and 17.8°C and was set on the camera each day. During January *E. europaeus* should not be reproductively active, and are typically hibernating (Reeve, 1994). Despite the artificially raised ambient temperature due to being housed inside, the shorter day lengths and their natural thermoregulatory cycle could result in core temperature readings being different to those identified by Fowler and Racey (1987 and 1990) during the summer and autumn months. To account for any potential difference in core temperatures caused by the time of year imaging of a further four males and four females, was carried out in the summer on 20/07/17. Due to the setup and policies of the rescue centre, individuals are released as soon as they are deemed healthy and fit for the wild, so images over consecutive days could not be obtained at any time other than during winter.

A further 8 individuals (5 males, 3 females) were sampled for eye surface temperature readings using the FLIR E60bx and compared to their skin fold axillary temperature. This was carried out whilst the individuals were undergoing their final health check by centre staff prior to release.

Rectal temperatures were obtained, using a digital thermometer, simultaneously with an IRT eye image using the FLIR E60bx for one adult male (02/08/17) and one juvenile male (07/09/17), at a veterinary practice, in order to assess the parity of these two measures. The rectal temperatures were taken by a registered veterinary nurse as the individuals were under anaesthetic for assessment and treatment of wounds. Due to the busy nature of veterinary practices and health and safety only two rectal temperature readings could be obtained whilst individuals were under anaesthetic. Although anaesthetic can affect core temperature (Aspinall, 2011) this measure was taken to allow a relative comparison of rectal and eye temperatures, not to show what a normal core temperature should be. Obtaining further rectal temperatures at the rescue centre was not appropriate or possible due to the difficulty in uncurling, and the stress it would cause an individual, which would have a negative impact upon their rehabilitation.

2.3.4 Hypothermia assessment

Eye surface temperature data were collected on 55 individuals (23 males, 32 females) between May and December 2016. Only individuals weighing 300g or over were sampled for this aspect of the study as, to date, studies have only examined the core temperatures of adults. This threshold excludes hoglets and small juveniles. IRT images were taken using all three models of infrared camera, multiple images of the face, focusing on the open eye were taken with each camera (refer to section 2.3.1). Staff diagnosis was recorded, independently of the cameras, whilst staff were handling and assessing new arrivals to the centre, before any treatment was administered. This diagnosis was based upon behavioural cues and touch, for continuity the same experienced staff member assessed all individuals to establish the 'staff diagnosis', based upon guidance provided by the centre on the different stages of hypothermia (Appendix 6). Date, time, individual ID (as given by the rescue centre), weight and conditions during transport (heat source provided or not) were recorded. Staff diagnosis of 'not hypothermic', 'mildly hypothermic',

‘hypothermic’, ‘high temperature’ was recorded alongside ambient temperature and humidity. .

There is no accepted hypothermia scale in veterinary literature, it is simply defined as a reduction in core temperature (Boden, 2005), however the treatment, specifically the duration of treatment, will depend on the level of reduction in core temperature. For the purposes of this study a hypothermia scale was retrospectively created as a way of categorising the temperatures documented in this study based on survival of those animals assessed.. This is a working scale, applicable to this data set, developed in consultation with Paul Thomas RCVS (Pers. comm., 2017) based upon previous research of *E. europaeus* core temperatures (34.7 - 36.2°C; Fowler and Racey, 1987) and the outcome of individuals within this study (survived or date of death and time elapsed since admission, e.g. within 24 hours of admission). Consideration was given in creating the scale to the small data set (eye temperatures n=55) making it hard to determine if those individuals who died did so due to hypothermia rather than other coincidental causes. Within the data set 27.4°C followed by 31.5°C were the lowest eye surface temperatures recorded of individuals who survived and went on to make a full recovery and return to the wild. Fourteen out of 17 individuals with eye temperatures below 30.0°C died, informing the lower end of the temperature scale.

Category	Temperature range
High temperature	≥36.3°C
Normal temperature	36.2-33.8°C
Mild hypothermia	33.7-32.0°C
Hypothermic	≤31.9°C

Table 2: Temperature / hypothermia classification scale applied to E. europapaeus admitted to Prickles and Paws Hedgehog Rescue Centre sampled in this study.

FLIR Tools was used to extract temperature data from the infrared images with ambient temperature set on the software (refer to section 2.3.1).

2.3.5 Statistical analysis

To test sensitivity of the three different model cameras linear regressions of IRT temperature against actual temperature were compared amongst cameras using Microsoft Excel. All other statistical analysis was carried out in Minitab 17. Baseline skin fold and eye temperature data was analysed using a Wilcoxon signed rank test to determine differences in the two temperature measurement methods. Axillary skin temperatures of males and females were analysed using a Man Whitney U test. Chi squared goodness of fit analysis was used to determine whether there was a difference between the staff diagnosis and FLIR E60bx diagnosis, using the FLIR E60bx values as the expected diagnosis. In order to assess the level of confidence in each of the cameras ability to accurately assess eye surface temperature of *E. europaeus,* as a proxy for core temperature, recorded temperatures from the three different camera models were compared using linear regressions in Microsoft Excel to establish equation of the line and intercept values. This data was further analysed using a non-parametric one-way repeated measures ANOVA to enable the testing of a rank order. A descending rank order of FLIR ONE, FLIR C2 and FLIR E60bx was applied based on results from the thermal calibration trial identifying differences in the cameras sensitivity and temperature readings.

2.3.6 Cost benefit analysis

A cost benefit analysis of the three infrared cameras trialled (FLIR E60bx, FLIR C2 and FLIR ONE) was created using a combination of results presented in this study, manufacturer information and wider knowledge. Costs were provided by Mathew Clavey, a FLIR representative on the 27 October, 2017.

2.4 Results

2.4.1 Thermal calibration

The FLIR E60bx and FLIR C2 cameras show minimal scatter around their lines, which appear almost parallel, the intercept values show a small difference of 0.7 (Figure 3). The FLIR ONE shows increased scatter around the line with a slightly smaller r^2 value (Figure 3). Readings were not consistently above or below the FLIR E60bx and FLIR C2 readings, but produced a negative intercept value, showing a notable difference in the slope of the line in comparison to the other two cameras. The thermal calibration 'tin trial' was repeated (Figure 4), using the same FLIR ONE as in the first trial ('FLIR ONE Original') and an additional FLIR ONE camera ('FLIR ONE New'). The results show distinct differences between the two FLIR ONE cameras, with the New camera producing a negative intercept and the Original camera producing a positive intercept. There was a considerable intercept difference of eight produced by the two FLIR ONE cameras, with the narrow temperature range of the diagnostic categories outlined in Table 2 this could be the difference between one or more categories.

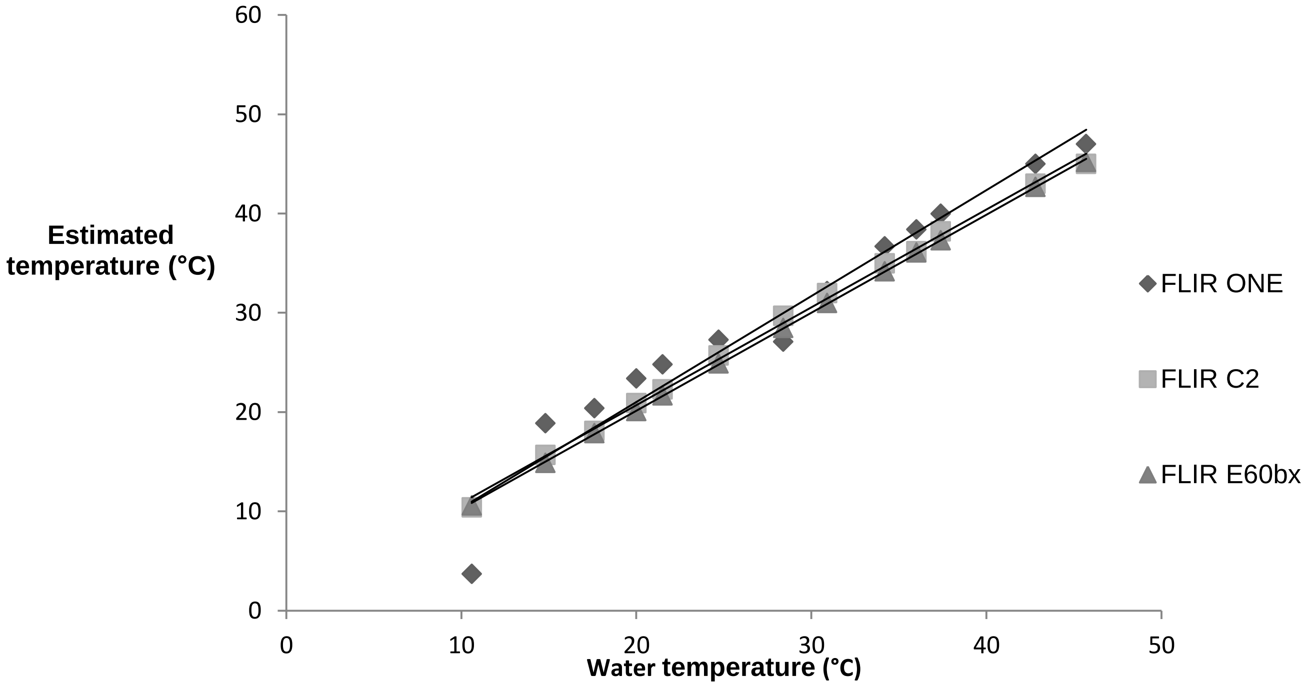


Figure 3: Water temperature and tape temperature, with linear regressions (n=13, for each regression), for the following infrared cameras: FLIR E60bx (y=0.99x + 0.38, R^2=1.0), FLIR C2 (y=0.99x + 1.001, R^2=1.0) and FLIR ONE (y=1.07x - 0.33, R^2=0.95).

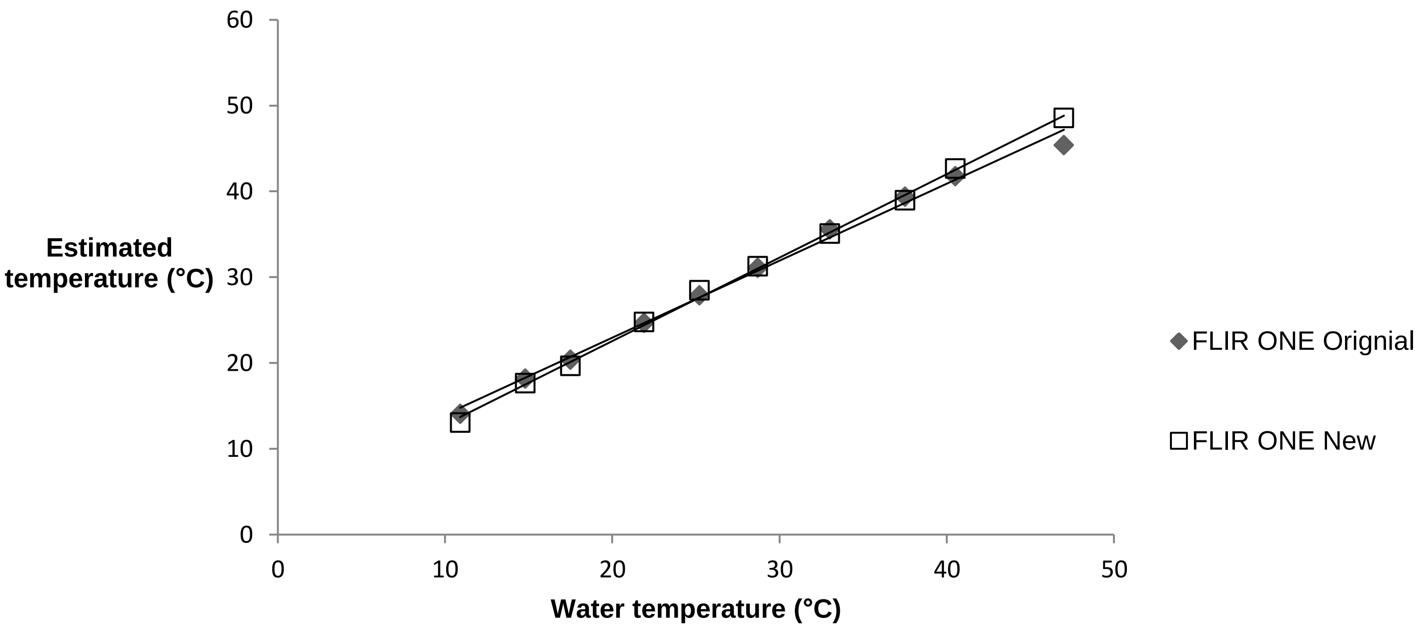


Figure 4: Water temperature and tape temperature with linear regressions for two FLIR ONE cameras (n=10 for each regression): FLIR ONE Original (y=0.90x + 5.02, R^2=1.0) and FLIR ONE New (y=0.97x – 3.11, R^2=1.0).

2.4.2 Base line temperature assessment

Infrared eye images taken over three days in January (2016) produced similar means of 34.50°C (SE.±0.15, n=5) for males and 34.37°C (SE.±0.21, n=5) for females (removing one anomalous result, on one day for one individual who was over 2°C lower on one of the three days of sampling: 34.25°C, SE.±0.18). The maximum range in temperatures over the three days was 1.8°C for males and 2.9°C (1.8°C removing the anomalous result) for females. A further four males and four females (July 2017) produced means of 34.65°C (SE.±0.10) and 34.86°C (SE.±0.13) respectively, showing no statistical difference between males and females (Mann Whitney U, W=14, p<0.30, n=4).

When sex was disregarded there was a significant difference between the eye and axillary skin temperature readings (Figure 5, Wilcoxon signed rank test, W=36, n=8, p<0.014). Axillary skin temperature was consistently below IRT eye temperature with a mean difference of 1.33°C (SE.±0.18), and minimal difference between males and females (0.03°C), this was not analysed due to uneven and small sample size.

Two individuals had rectal and IRT eye temperatures taken whilst under anaesthetic which demonstrated only a ±0.1°C difference between the two methods in both individuals (Individual 1: rectal: 34.9°C, eye: 34.8°C, Individual 2: rectal: 35.3°C, eye: 35.4°C).

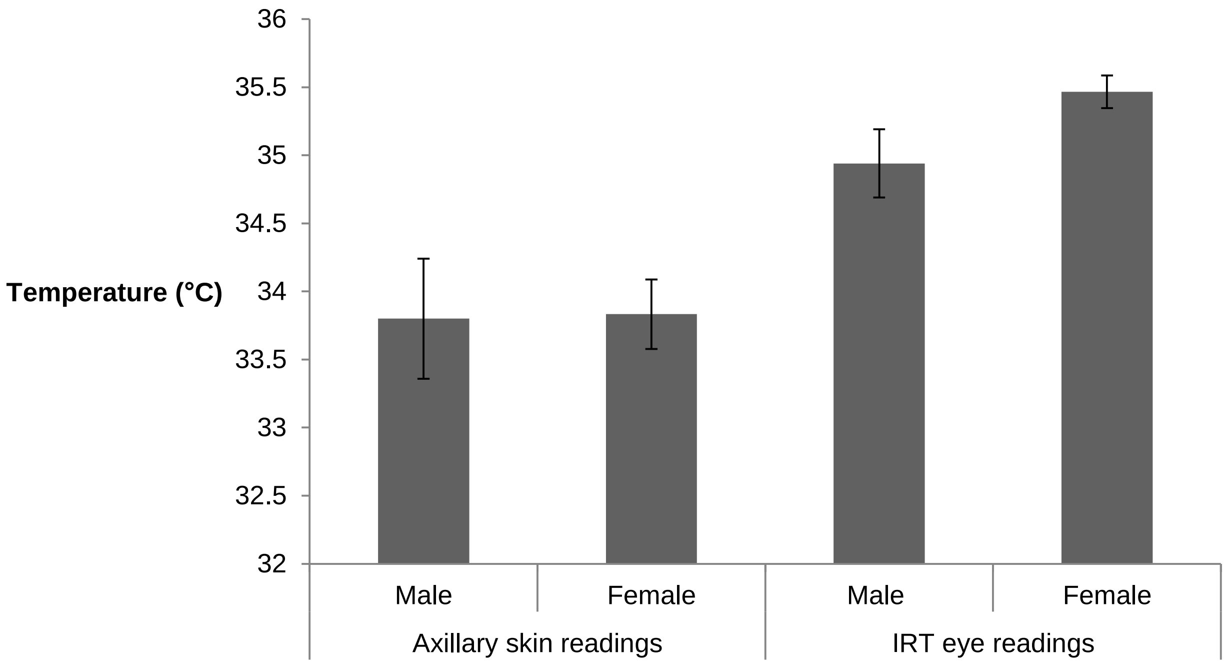


Figure 5: Mean axillary skin temperature and IRT eye surface temperature readings, taken using the FLIR E60bx, for healthy male (n=5) and female (n=3) *E. europaeus* (±SE).

2.4.3 Hypothermia assessment

Of the 55 individuals sampled 35 died or were euthanised, eight of these could be attributed to causes such as haemorrhage, internal bleeding or fractures, although shock and hypothermia will likely also have played a role. Twenty of those that died did so within 24 hours of admission, six within 48 hours and five within 72 hours.

Thirty two individuals were diagnosed, using the FLIR E60bx, as having ‘mild hypothermia’ or as ‘hypothermic’ based upon the criteria outlined in Table 1, of these 24 died within 72 hours of admission (Table 3). It should be noted that treatment was based upon the staff diagnosis not the camera diagnosis and no post-mortems were carried out to establish cause of death so a direct link between misdiagnosis and survival cannot be made. Figure 6 compares staff diagnosis (based upon behavioural cues and symptoms outlined in Appendix 6) to the FLIR E60bx diagnosis based on the categories in Table 2. The category ‘high temperature’ or hyperthermia shows no difference in number diagnosed. There were only two individuals within this category and both had infected wounds which greatly influenced staff diagnosis. All other categories show differences and overall a statistically significant difference between the number of individuals diagnosed within each category by staff compared to the expected diagnosis recorded through the FLIR E60bx with staff diagnosing more as ‘normal temperature’ and ‘mild hypothermia’ but less as ‘hypothermic’

(n=55, df=3, X^2=11.43, p<0.010). This is based upon the assumption that the FLIR E60bx is more accurate at diagnosing the level of hypothermia based on eye temperature as an accepted proxy for core temperature, evidenced by the FLIR E60bx compared to rectal temperature, rather than behavioural cues and symptoms observable by staff.

Time of death after admittance (hours)	Sample Size	Misdiagnosed as normal instead of mild	Misdiagnosed as mild instead of hypothermic	Diagnosed correctly
24	17	1	4	12
48	6	0	3	3
72	5	0	1	4
Survived	20	4	1	14

Table 3: Staff diagnosis in comparison to FLIR E60bx diagnosis of *E. europaeus* (assumed as the more accurate diagnosis here) and outcome: survived / died. The table excludes 3 individuals that died over 72 hours after admission (1 diagnosed correctly, 1 misdiagnosed as mild instead of normal and 1 misdiagnosed as mild instead of hypothermic) and 4 individuals which were euthanised (all diagnosed correctly).

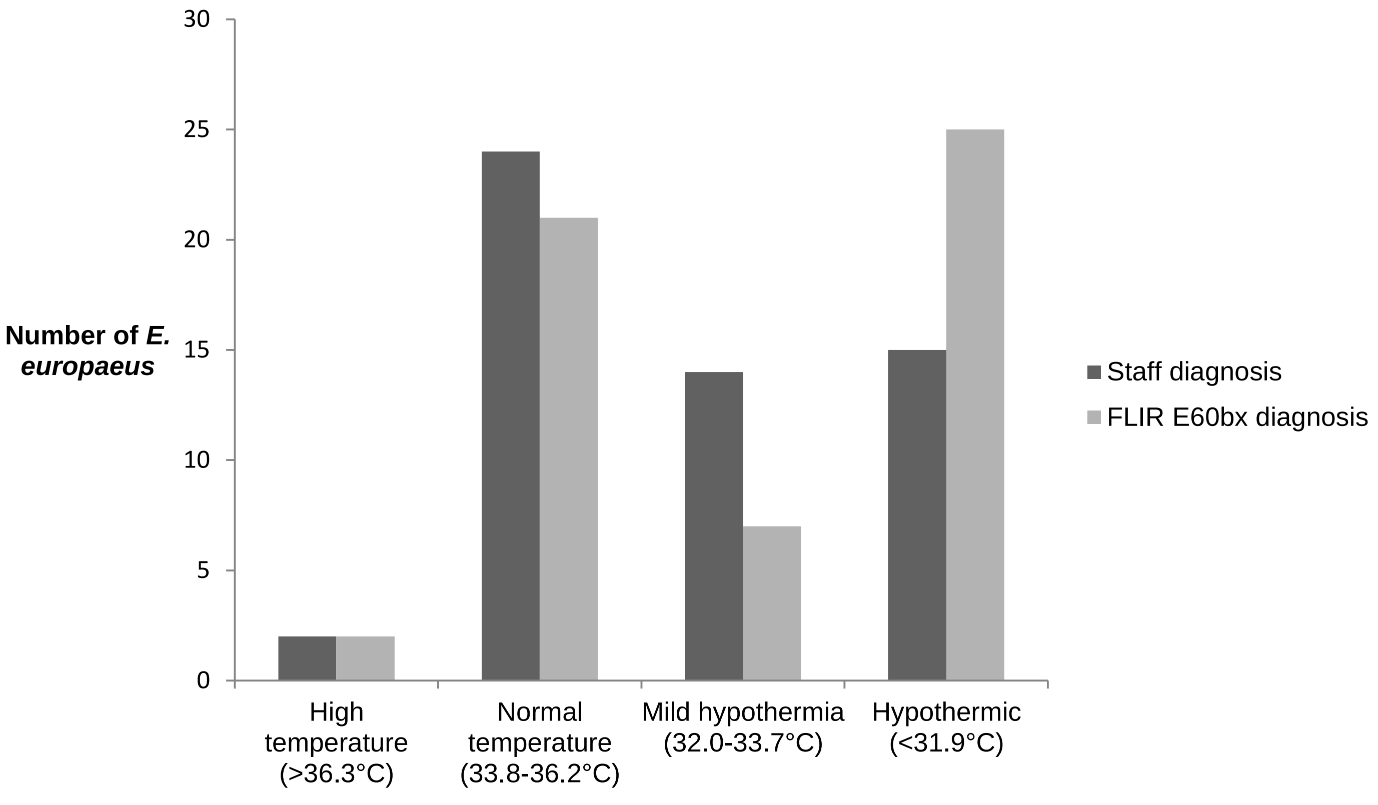


Figure 6: Staff diagnosis (based upon behavioural cues and symptoms outlined in Appendix 6) and FLIR E60bx temperature diagnosis (based upon eye surface temperature as a proxy for core temperature) of newly admitted *E. europaeus* to Prickles and Paws Hedgehog Rescue (n=55).

The FLIR E60bx diagnosis consists of four categories (Table 2): these categories demonstrate the increased level of detail and small incremental differences that the cameras can identify in diagnosis when compared to staff diagnosis. Within 55 individuals the majority of individuals fell within two categories with most in ‘hypothermic’ followed by ‘normal temperature’ and the smallest category ‘high temperature’ (Figure 6). Determining that an individual has a normal temperature is just as useful as determining that it is hyperthermic or hypothermic in a rehabilitation setting.

Hypothermia assessment readings from the three cameras were compared against each other (Figure 7, 8 and 9). There is a clear reduction in scattering around the line in Figure 7, which compared the FLIR E60bx and FLIR C2, and a lower intercept value of – 0.81 compared to -1.3 and -5.23 for figures 8 and 9, which compares both the FLIR E60bx and FLIR C2 with the FLIR, suggesting that the FLIR ONE camera is responsible for a greater proportion of the scattering which in Figure 8 is more variable in the higher and lower temperature ranges.

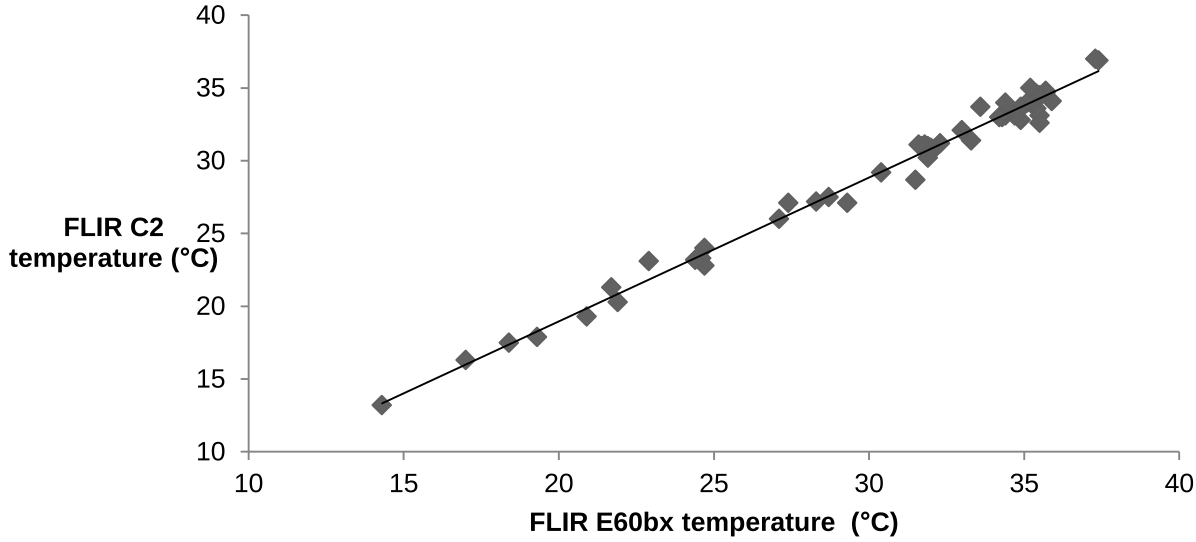


Figure 7: FLIR E60bx and FLIR C2 hypothermia assessment readings of 55 newly admitted *E. europaeus*, to Prickles and Paws Hedgehog Rescue ,with linear regression, (y= 0.99x – 0.81 R^2 = 0.99).

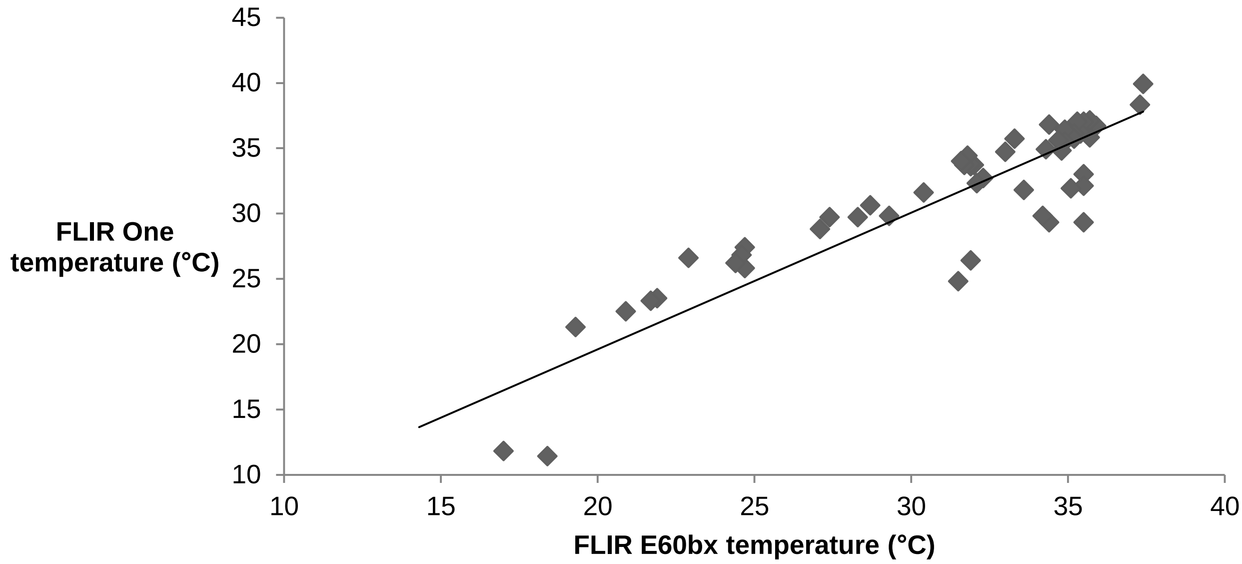


Figure 8: FLIR E60bx and FLIR ONE hypothermia assessment readings of 55 newly admitted *E. europaeus,* to Prickles and Paws Hedgehog Rescue ,with linear regression, (y= 1.05x – 1.3 R^2 = 0.82).

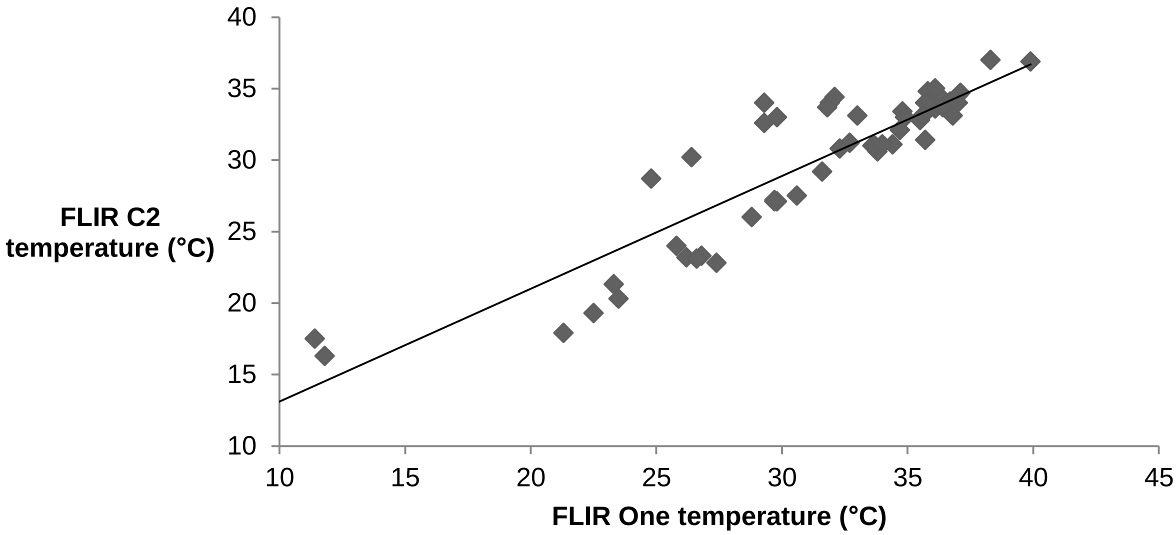


Figure 9: FLIR One and FLIR C2 hypothermia assessment readings of 55 newly admitted *E. europaeus,* to Prickles and Paws Hedgehog Rescue ,with linear regression, (y= 0.79x – 5.23 R^2 = 0.84)

The differences between the three camera readings was further analysed, there was a statistically significant difference between eye temperatures recorded by the three infrared cameras for each individual sampled (n=53, H=43.19, df=2, *p*<0.001). The rank order of FLIR ONE reading higher temperatures, followed by the FLIR C2 and then the FLIR E60bx reading lowest temperatures, as indicated by the thermal calibration trial (Figure 3) was however not significant (Z= -1.46, *p*=0.9279) suggesting that the order was not consistent throughout the data (Appendix 7). The FLIR ONE varied from being lower or higher in comparison to the other two cameras with the majority of readings higher than the other two cameras. The readings from the FLIR E60bx were generally higher than those from the FLIR C2, a pattern which was not evident in the thermal calibration trial, although the results were generally very close with a mean difference of -1.11°C ($\pm$0.067 SE) between the two cameras (Table 4). This difference was not consistent, two readings were higher than the FLIR C2.

Category	Sample size	FLIR E60bx and FLIR C2		FLIR E60bx and FLIR One		FLIR C2 and FLIR One	
		Mean +SE	Range (°C)	Mean +SE	Range (°C)	Mean +SE	Range (°C)
All data	55	-1.11°C +0.067	-2.9 to 0.2	0.22°C +0.283	-7.0 to 3.7	1.31°C +0.269	-6.1 to -4.6
High Temperature (>36.3°C)	2	-0.4°C +0.1	-0.3 to -0.5	1.75°C +0.75	1.0 to 2.5	2.15°C +0.85	1.3 to 3.0
Normal Temperature (36.2 – 33.8°C)	21	-1.33°C +0.135	-2.9 to -0.2	-0.50°C +0.548	-6.2 to 2.4	0.84°C +0.534	-4.7 to 3.7
Mildly hypothermic (33.7 – 32.0°C)	7	-1.03°C +0.225	-1.9 to 0.1	0.73°C +0.650	-1.3 to 3.1	1.76°C +0.572	-0.3 to 4.4
Hypothermic (<31.9°C)	25	-1.13°C +0.127	-2.8 to 0.2	0.30°C +0.667	-7.0 to 3.7	1.43°C +0.635	-6.1 to 4.6

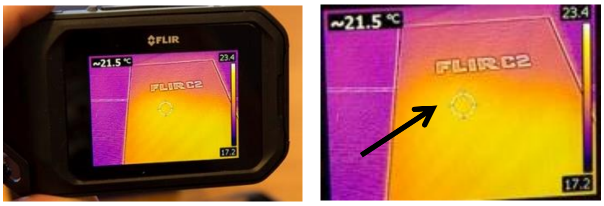
Table 4: Mean difference between infrared cameras (+SE). Raw data of hypothermia diagnosis, based upon the E60bx being the most accurate, the category an individual has been placed in is determined by the E60bx result and shows a comparison to the other two cameras.

When the 53 individuals (excluding the two ‘high temperature’ individuals due to the small sample size) were split into the three diagnostic categories (normal temperature, mild hypothermia, hypothermic), the significant difference between the three cameras remains, with the exception of the ‘mild hypothermia’ category where there was no significant difference between the three cameras (Table 5). This may be due to the narrow temperature range (1.7°C) of the category. The rank order for the three cameras within each temperature category was not statistically significant, again with no consistent pattern, but the FLIR C2 was generally lower than the FLIR E60bx and the FLIR ONE generally higher. Summaries of the cost and capabilities of the three model cameras for the application of hypothermia diagnosis, tested here, are presented in Table 6.

Diagnostic category	N (Individuals within each category)	H	Df (Three cameras)	*P* (Regardless of rank order)	Z	*p* (Camera rank order)
All	53	43.19	2	0.0001	-1.46	0.9279
Normal temperature	21	16.36	2	0.0003	-0.31	0.6219
Mild hypothermia	7	3.71	2	0.1561	-0.53	0.7035
Hypothermia	25	24.08	2	0.0001	-1.56	0.9401

Table 5: One-way repeated measures non-parametric ANOVA results testing eye surface temperature of newly admitted E. europaeus as recorded by, and in the descending rank order of, FLIR ONE, FLIR C2 and FLIR E60bx. (P(camera rank order) represents the P value produced from testing of the stated rank order) The category an individual has been placed in is determined by the E60bx result, based upon assumption that the E60bx being the most accurate.

Camera	Cost	Specifications	Usability	Application
FLIR E60bx	£5,000 Requires a humidity and temperature metre to allow these measurements to be set on the camera Requires equipment to determine RAT – ‘foil method’ (See page 23)	Thermal sensitivity <0.045°C at 30°C, sensitivities over a greater temperature range are not published by the manufacturer Temperature range: -20 to 120°C IR sensor 240 x 320 pixels, allowing longer range accurate images and high resolution Thermal and visual images Emissivity, humidity, RAT and ambient temperature can be set using custom values	Thermal and visual images. Hand held with touch screen, spot tools allow a temperature to be taken without needing to download an image into FLIR Tools for analysis (a freely downloadable software associated with all FLIR IR cameras) 4hr battery life	Using eye surface temperature ±0.1°C different to rectal temperature (n=2) Can be used in darkness so when an individual is reluctant to uncurl can be observed in their hutch and allowed to uncurl in their own time until an eye is visible. Digital images can be switched off so no flash, resolution is clear enough to accurately pinpoint the eye to obtain a reading.
FLIR C2	£550 Requires equipment to determine RAT	Thermal sensitivity <0.10°C Temperature range: -20 to	Thermal and visual images Small and portable, strap so	A mean difference of 1.12°C (±0.067 SE) lower than the FLIR E60bx temperature readings in this study

	– ‘foil method’ (See page 23) Ambient temperature metre needed so that this can be factored into analysis in FLIR Tools Need access to FLIR Tools software which is free but needs to be run on a computer, laptop or smart phone. Access to Microsoft Excel to develop correction protocol	120°C IR sensor 80 x 60 pixels (The same IR sensor used in the FLIR ONE Camera) Thermal and visual images Emissivity and reflected apparent temperature can be set using custom values Camera model calibrated on mass (one cameras calibration given to many)	can be worn around neck and protective casing 2hr battery life (rechargeable by micro USB connection) Has a circle in the centre of the screen which must be filled with the area you want to obtain the temperature for (see image below), this guide allows sufficient pixels to produce an accurate temperature of the area.  (Images from Frazel, 2018, arrow indicates circle)	with a range of small range of -1.29°C to +0.2°C difference. Can be used in darkness so when an individual is reluctant to uncurl can be observed in their hutch and allowed to uncurl in their own time until an eye is visible. Thermal image is clear enough to pinpoint eye temperature, MSX technology less accurate with short distance from the individual.
FLIR One	£250 2 versions – one for android, one for iphone (independent of the phones camera, the plug in contain both a digital and IR	Thermal sensitivity not stated IR sensor 80x60 pixels (The same IR sensor used in the FLIR C2 Camera) IR sensor Camera model calibrated on mass	Thermal and visual images Small, cannot be used with some phone cases on as the width of the case prevents connection, there are two models, one for android one for iPhone	A mean difference of 0.22°C (±0.283 SE) higher than the FLIR E60bx temperature readings in this study with a range of -7.0°C to 3.7°C difference. Can be used in darkness so when an individual is reluctant to uncurl can be

camera, will work on any smart phone) Requires equipment to determine RAT – ‘foil method’ (See page 23) and an ambient temperature metre needed so that this can be factored into analysis in FLIR Tools Need access to FLIR Tools software which is free but needs to be run on a computer, laptop or smart phone, the emissivity can be altered in this analysis. Access to Microsoft Excel to develop correction protocol	(one cameras calibration given to many) Pre-set emissivity values only (0.95 is the closest to the 0.98 used in this study), no setting for ambient temperature or RAT. Once downloaded into FLIR Tools the emissivity can be adjusted and the RAT set.	Connection to phone slightly fragile, needs to be used with care 1 hour battery life (does not charge off the phone) No circle to determine sufficient pixel number to obtain accurate temperature reading When close to an object the MSX technology (overlay of thermal and IR image so create object outlines) does not work effectively with the visual and thermal overlay not aligned.	observed in their hutch and allowed to uncurl in their own time until an eye is visible. Thermal image is clear enough to pinpoint eye temperature. MSX technology less accurate with short distance have to go by the thermal colour spots rather than the outline MSX technology puts onto the image as they are misaligned at such short distances (3m is optimal for MSX technology but this is not close enough to obtain an eye image)

Table 6: Cost benefit analysis of the FLIR E60bx, FLIR C2 and FLIR ONE infrared cameras for the application of eye surface temperature imaging to allow hypothermia diagnosis in *E. europaeus.*

2.5 Discussion

This study provides further evidence for the continued use and exploration of IRT with animals, wildlife in particular, and provides evidence to influence rescue centre protocols. The FLIR E60bx demonstrates increased accuracy, compared to staff assessment alone, in assessing newly admitted *E. europaeus* to rescue centres and diagnosing hypothermia. There was a statistically significant difference between the staff diagnosis and camera diagnosis. The narrow diagnostic categories of the camera could not be matched by staff; many individuals which the camera placed in the mild hypothermia category were misdiagnosed as normal temperature by staff, whilst those diagnosed as having mild hypothermia by staff were deemed hypothermic by the camera. This application of the FLIR E60bx therefore has important implications for treatment protocols, preventing deterioration and improving the accuracy of initial diagnosis, ultimately increasing survival rates.

2.5.1 Thermal calibration

Of the three cameras assessed, the FLIR E60bx was the most accurate and consistent, followed by the FLIR C2 and FLIR ONE. The thermal calibration trial was designed to establish any variation between the different models of camera before any images were taken of live *E. europaeus* with the view to developing correction protocols. The regressions demonstrated similar results recorded by the FLIR E60bx and FLIR C2 but inconsistent results by both FLIR ONE cameras tested. This was unexpected considering that the FLIR ONE and FLIR C2 share the same IR sensor of 60x80 (FLIR b, 2017; FLIR c, 2017, Appendix 4 and 5) and analysis in FLIR Tools software accounts for differences in ambient temperature and RAT. The FLIR ONE recorded hotter temperatures than the FLIR C2 and FLIR E60bx between 25°C and 35°C as well as a single high reading at 10°C but all other readings were below the FLIR C2 and FLIR E60bx. The lack of a pattern and inconsistent results between the two FLIR ONE cameras, tested simultaneously, meant that a correction was not appropriate and would likely not have been transferable. A representative from FLIR confirmed that the FLIR ONE and the FLIR C2 camera models are calibrated on mass, with one cameras calibration being given to x number of cameras (M Clavey 2017, Pers. comm., 27 October), likely explaining the variation between the two FLIR ONE's. Further research comparing FLIR C2 cameras would likely demonstrate the same pattern, therefore calibration or correction factors can only be applied to an individual camera and not a standard across all cameras of the same model. A full cost benefit analysis of the three infrared cameras trialled is provided in Table 6.

2.5.2 Baseline temperatures assessment and hypothermia

Previous studies have already demonstrated the effectiveness of IRT in temperature assessment of animals, with Herborn *et al.* (2015) commenting that with the present cost-effective availability of IRT technology, this non-invasive method of temperature assessment in unrestrained animals has the potential to become common practice in pure and applied research. In this study only two individuals were able to have simultaneous eye (IRT) and rectal temperatures recorded, with both readings of the FLIR E60bx eye temperature being only ±0.1°C of the 'gold standard' veterinary rectal temperature recorded (Sousa *et al.,* 2013). This demonstrates a high level of accuracy and is supported by Pérez de Diego *et al.* (2013) who reported eye and rectal temperature to be significantly correlated in cows, with a mean difference between eye surface temperature and rectal temperature of 1.46°C ±0.05°C (IRT eye lower than rectal temperature). This relationship is likely transferable to all mammals, suggesting that IRT could replace the invasive rectal temperature (likely with a correction factor for different model cameras as well as the species of study) as a measure of core temperature. With a larger sample size greater confidence could be given to the accuracy of the cameras when compared to rectal temperature. Baseline temperature studies of healthy individuals sampled before release from the rescue centre using the FLIR E60bx, demonstrated eye temperatures at the lower end of the previously reported core range (Fowler and Racey, 1987 and 1990). This is not unexpected as Fowler and Racey (1990) reported that *E. europaeus* demonstrate a clear circadian rhythm with maximum daily temperatures at midnight and minimum at midday, and the majority of healthy individuals were measured midmorning, to coincide with the centres weighing/handling regime. Factors such as individuals blinking and openness of the eye must also be considered when interpreting this data, images were taken consecutively, not simultaneously, allowing for blinking to take place which may have affected temperatures recorded.

All three camera models were tested for the hypothermia diagnostic application. Statistical analysis confirmed that there was a significant difference between the eye temperatures recorded by the three different cameras for the 55 individuals assessed for hypothermia. The accuracy and consistency of the FLIR E60bx, as demonstrated by the trial, made it the ideal camera to assess new *E. europaeus* eye temperature in comparison to staff diagnosis. But it also shows that the FLIR C2 used in this trial was still a useful tool, almost consistently 1°C lower that the FLIR E60bx and giving a thermal profile of the individual, to combine with staff diagnosis (See Table 7). Although this study did not evidence it, the cameras can also be used to monitor changes in eye temperature after admission in response to the warming process (incubator or heat pad and a saline and electrolyte solution injected subcutaneously), as each individual will likely respond slightly differently due to factors which are often unknown, such as age, body condition, injury or internal trauma and length of time they have been hypothermic. Despite the FLIR E60bx being the most appropriate, all three of the cameras would allow monitoring in response to treatment, as a relative difference to the initial temperature recorded can be identified, this allows treatment to be tailored and continued until they have reached normal core temperature which could be judged by multiple similar readings after treatment combined with staff diagnostic experience.

The FLIR E60bx however, can establish the closest reading to actual core temperature (through eye surface temperature) on admission, which can be easily and non-invasively monitored periodically over the next 24 hours. An accurate initial core temperature reduces the risk of mildly hypothermic individuals being missed by staff and only diagnosed once they have further deteriorated; it allows immediate and appropriate treatment for their condition on admission to the rescue centre. This has the potential to become a widespread tool for assessing *E. europaeus.* Staff diagnosis was significantly different to the FLIR E60bx diagnosis (Figure 6). With the camera taken to be the more accurate diagnostic tool, staff misdiagnosed five of the seven actual cases of 'mild hypothermia' as 'normal temperature'. The majority of those which should have fallen under the 'hypothermic' category according to the camera were diagnosed by staff as having 'mild hypothermia'. The temperature range of the 'mild hypothermia' category is very small at only 1.7°C, making accurate diagnosis of this category using no temperature measurement tools very difficult for staff. Staff guidelines (Appendix 6) are unlikely to be able to diagnose accurately within such a narrow category as defined in Table 2, however based upon the camera results and survival of study individuals the small range is appropriate and with application of IRT may allow further refinement of diagnosis and treatment.

With the ability to non-invasively, accurately assess core temperature and diagnose hypothermia the door is opened to reviewing hypothermia treatment protocols. Out of the 55 individuals assessed 24 individuals died within 72 hours of admission. Post-mortem examinations were not carried out on any of the study individuals so cause of death cannot be established although staff can speculate based on their condition. The number of deaths within those diagnosed correctly evidences that there are other factors than just hypothermia and also calls into question the effectiveness of hypothermia treatment, The severity of hypothermia potentially being misdiagnosed in some individuals combined with non-specific treatment (warm fluids injected and a source of heat being provided e.g. heat pad) could be a factor leading to these deaths. Having a method to accurately assess core temperature would therefore allow development of a specific treatment/rewarming protocol slowly raising temperature in small increments based on information from medical literature and the initial core temperature diagnosed..

In medical literature it is widely accepted that many deaths occur due to complications after low temperature has been successfully treated/raised (van der Ploeg *et al.,* 2010). If this is found in humans, it will likely apply to other mammal species including *E. europaeus*. Many *E. europaeus* appeared to have been successfully treated, core temperatures restored to within normal range before deterioration and death up to 72 hours later. Van der Ploeg *et al.* (2010) refer to a human study by Bierens *et al.,* (1995) which reported that 60% of nonsurvivors died after more than two days and concluded that late complications were most likely the cause. Van der Ploeg *et al.* (2010) recorded 24 different complications in their study of 84 patients admitted to hospitals suffering from hypothermia (causes categorised into: immersion, submersion, outdoor exposure, indoor exposure). The complications include pulmonary oedema, liver and kidney failure, neurological problems (cerebral ischemia and convulsions) and infections such as renal, pulmonary and general sepsis). These complications and conditions may also occur in *E. europaeus* and therefore could explain some fatalities in the present study. When *E. europaeus* enter hibernation, lowering their core temperature dramatically, they periodically arouse spontaneously to reduce the risk of oxidative stress and neuronal damage (Humphries *et al.,* 2003). These complications likely occur during hypothermia, which unlike hibernation where the body goes through preparation processes (such as reduction in blood insulin levels, Reeve, 1994), is not voluntary. This supports the assumption that there can be fatal complications as a result of hypothermia post treatment, likely a combination of the length of time an individual is hypothermic and the rewarming process.

The next logical step is to combine IRT in both diagnosis and treatment to try and reduce these complications and increase survival rates. Medical studies have examined the use of hypothermia therapeutically in piglets and human babies to treat birth asphyxia and for its neuroprotective effect, however rapid rewarming exacerbated neuronal/axonal damage in contrast to slow rewarming which enhanced neuroprotective effects which actually extended to cerebral microcirculation (Thoresen *et al.,* 1995; Suehiro *et al.,* 2004). This suggests that the speed of the warming process is key to reducing complications and protecting neuronal function. A study by Maxwell *et al.* (2005) on male albino guinea pigs concluded that re-warming faster than 1°C every 40 minutes following mild post-traumatic hypothermia induces secondary axonal pathology. Mosalli (2012) recommend a rewarming rate of 0.5°C per hour for human infants with hypoxic-ischemic encephalopathy with a minimum rewarming period of 4 hours. With no previous way to accurately measure core temperature in *E. europaeus* there appears to have been no research on hypothermia treatment and rewarming protocols for *E. europaeus*. The only known advice available to rescue centres is provided by Bullen (2010) who refers to warming too quickly causing shock. Medical papers also refer to rewarming shock, which is characterized by low cardiac output and a sudden fall in peripheral arterial pressure (Kondratiev *et al.,* 2006). It appears that current treatment for hypothermia in *E. europaeus* is not evidence-based since rescue centres have previously been unable to establish accurate core temperatures. IRT could pave the way for developing rewarming protocols and increasing long-term success rates of hypothermia treatment, and when the number admitted to rescue centres suffering from hypothermia are considered this could positively affect *E. europaeus* conservation.

Chapter 3: Hibernation

3.1 Introduction

3.1.1 *Erinaceus europaeus* hibernation

Hibernation is an adaptive mechanism which allows homeothermic animals to conserve energy and overcome unfavourable environmental conditions, low temperatures and reduced food availability, during winter (Reeve, 1994). It is defined as a state of inactivity and metabolic depression to conserve energy during adverse conditions (Reeve, 1994). During hibernation *E. europaeus* heart rate can fall to five beats per minute and respiration rate to 13 breaths per minute (Pfäffle, 2010). Blood chemistry changes, with reduced platelets levels, which prevents clotting, as well as reduced insulin levels to drastically slow metabolism and a slow increase in red blood cell count and haematocrit (ratio of the volume of red blood cells to the total volume of blood) over the hibernation period (Reeve, 1994; de Vrij and Henning, 2015). *Erinaceus europaeus* commonly hibernate in the UK between the months of October and April, with climate, individual condition and sex influencing date and duration of hibernation periods (Reeve, 1994). Hibernation is triggered by a combination of environmental factors, including photoperiod, body condition, food availability and consistently low temperatures, commonly quoted as 5°C and below, although studies indicate that the 'trigger' value varies across Europe (Reeve, 1994). *E. europaeus* body temperature is variable during hibernation, Fowler and Racey (1990) reported that body temperature significantly correlated with ambient temperature ($p < 0.01$, $r = 0.949$). They recorded body temperatures between 5.2 and 17.7°C throughout the hibernation period, a range of over 14°C (Fowler and Racey, 1990).

Surviving hibernation requires considerable pre-immergent energy storage (Humphries *et al.* 2003), in *E. europaeus* this is in the form of body fat (Reeve, 1994). A study on white-tailed prairie dogs, *Cynomys leucurus,* and black-tail prairie dogs, *Cynomys ludovicianus,* identified that body protein can, in some circumstances, serve as a minor hibernation energy source (Harlow, 1995). This is unlikely, or may only occur in underweight *E. europaeus* as individuals can lay down large adipose reserves. Adipose tissue is the main energy storage tissue in mammals as it is compact and has a high energy yield for its weight (39.33 kJ g^{-1}); *E. europaeus* rely upon stores of both white and brown adipose tissue stores (Reeve, 1994). White adipose is stored subcutaneously and among the mesenteries of the abdominal cavity, providing a long term energy store (Reeve, 1994). *Erinaceus europaeus* also build up stores of brown adipose, which are symmetrically distributed, the largest lobes found in the axillary region (Reeve, 1994). Brown adipose tissue has long been linked to heat production in hibernating species, including the bat, *Eptesicus fuscus,* (Smalley and Dryer, 1963). More recent research has confirmed the important physiological role brown adipose tissue plays in arousal from hibernation, specifically in non-shivering thermogenesis (Kitao and Hashimoto, 2012). Non-shivering thermogenesis is defined as a metabolic process located primarily in brown adipose tissue and controlled by the activity of the sympathetic nervous supply of this tissue (Kitao and Hashimoto, 2012). In *E. europaeus* the tissue is involved in entry into and arousal from hibernation (Himms-Hagen, 1984). Reeve (1994) refers to a study by Girardier (1983), which calculated the tissue can produce up to 400 W kg^{-1} of heat.

Several studies have looked at the overall energy requirements surrounding hibernation. It is estimated *E. europaeus* lose 0.2% of their original body weight for each day that is spent in hibernation (between 0.8-2.0g day^{-1}, Wroot, 1984). Jensen (2004) recorded a mean weight loss during hibernation of 22.1% for juveniles and 30.2% for adult females. Spontaneous arousal, sometimes referred to as periodic arousal, is the term given to a brief awakening from hibernation. All mammals periodically arouse to normal body temperature during hibernation (Humphries *et al.,* 2003). Studies of other mammal species indicate that spontaneous arousal from hibernation is energetically expensive with up to 75% of total energy requirement for mammals during hibernation being associated with arousals (Thomas and Geiser, 1997). Considering how energetically expensive arousal during hibernation appears to be, its exact function and effects remains poorly understood.

The function of arousals is often speculated to involve recovery from physiological costs accrued during metabolic depression, which Humphries *et al.* (2003) suggest include oxidative stress, reduced immunocompetence and neuronal tissue damage. This could explain why many *E. europaeus* arousals do not involve leaving the nest. Walhovd (1979) observed multiple spontaneous increases in nest temperature throughout their study, on average an increase of 18°C, with no departure from the nest. These arousals were termed partial arousals. Other studies report that arousals in *E. europaeus* can last for several days and involve changing nest, with an average of two nests and a maximum of four used during hibernation (Reeve, 1994; Jensen, 2004). Mean arousal duration of *E. europaeus* studied in outdoor hibernacula can last between 34-44 hours (Walhovd, 1979). Frequency of spontaneous arousal in *E. europaeus* has been recorded at 2.9 per month (Dmi'el and Schwarz, 1984, 29 individuals studied). Other similar figures are reported, yet demonstrating a large range, with 3-15 days between arousals and a total during the hibernation period of 12-

18 days (Walhovd, 1979). Reeve (1994) refers to a study by Kristoffersson and Soivio (1964) who report spontaneous arousal occurring on average every 7-11 days. These figures show a large variation in frequencies, which could be due to differing methodologies and environmental conditions, however it could also demonstrate variation among individuals and initial hibernation weights. This variation among individuals will have implications for the husbandry, specifically weighing and monitoring, of *E. europaeus* at rescue centres during winter months especially as Millineaux and Keeble (2016) refer to hibernation as the single greatest mortality factor for the species with up to 70% of young *E. europaeus* dying in their first winter.

Many *E. europaeus* are overwintered or hibernate at rescue centres (Stocker 2005; Bullen, 2010). Bullen (2010) recommends individuals which have not reached 600g, (considered a safe weight for hibernation), are not released before winter and instead allowed to reach this optimum weight and hibernate in the rescue centre. In the UK, Morris (2014) considers 450g the minimum body mass sufficient for an individual to hibernate, whilst Jensen (2004) reports 513g from a study in a Danish rural area. Evidence suggests handling or disturbance can artificially induce arousal, which has unknown effects upon weight loss (Pajunen, 1984; Reeve, 1994). With large numbers of the species admitted to rescue centres and many being kept over winter and potentially hibernating, the relationships between spontaneous arousal frequency and weight loss needs further exploration to ensure appropriate levels of weighing/disturbance, allowing a more natural hibernation period for them. . Weight loss, artificial hibernacula and different conditions in rescue centres may alter minimum 'safe' weights for hibernation which have already been observed and established in *E. europaeus* hibernating naturally in the wild.

3.1.2 IRT specific skin methodology and application

Infrared methodology has been evaluated with the application of the technology to skin temperature measurement. IRT requires bare skin (Herborn *et al.,* 2015); hair or feathers significantly affect the temperature on the outer surface of the body (Cilulko *et al.,* 2013), which is why Ludwig *et al.* (2007) had to shave a portion of the head on rabbits to obtain a skin temperature reading using IRT. Species which have thick fur coats, such as sheep or llamas, are weakly visible in thermograms (Cilulko *et al.,* 2013). Some mammals, such as seals and hippopotamus lack the typical layer of fur which acts as insulation and instead exhibit an extensive subcutaneous fat layer which provides the necessary insulation (Tattersall and Cadena, 2010). Different skin temperatures between adult and juvenile hippopotamus are evident from thermal imaging;adults with fatty insulation demonstrate relatively cool skin with the exception of the inner surfaces of the ear (Tattersall and Cadena, 2010). Soerensen and Pedersen (2015) refer to the correlation between core body temperature and skin temperature being highly dependent on the exact location the skin measure was taken. Layers of subcutaneous fat have the potential to affect skin temperature readings in all mammals (Cilulko *et al.,* 2010); this should be considered in any thermal imaging of *E. europaeus* as their subcutaneous fat layer thickens in the approach to winter and hibernation (Reeve, 1994). Skin temperature is lower at low ambient temperatures due to the fat insulation, while warmer ambient temperatures rapidly increase skin temperature due to vaso-controlled thermoregulation supplying more blood to the skin, so the environment must also be considered an important factor in skin temperature thermal imaging (Soerensen and Pedersen, 2015). The other main factor recorded as affecting skin temperature is stress. Herborn *et al.* (2015) used IRT in assessing stress in hens, *Gallus gallus,* finding skin temperature changes to be an accurate measure of stress, with acute stress triggering vasoconstriction, causing rapid, short-term drop in skin temperature, a result likely seen across all homeotherms.

Skin temperature has also been studied in several hibernating species including: 13-lined ground squirrels, *Spermophilus tridecimlineatus* and yellow-bellied marmots, *Marmota flaviventris* (Phillips and Heath, 2004), garden dormice, *Eliomys quercinus L.* (Pajunen, 1984), and woodchuck, *Marmota monax* (Phillips and Heath, 2001). The more recent studies have utilised non-invasive IRT, whilst Phillips and Heath (2004) corroborated the results obtained by IRT with implanted data loggers. The studies have analysed various anatomical areas and body surface temperature during hibernation, or arousal, but these have been carried out in controlled laboratory conditions (Pajunen, 1984; Phillips and Heath, 2001; Phillips and Heath, 2004). The natural hibernation of individuals, under natural or wild environmental conditions remains uninvestigated, likely due to logistics of using IRT within a wild nest or hibernaculum. A rescue centre setting with natural environmental temperature perhaps offers a new area for insight and study which could allow non-invasive monitoring of the hibernation process in rescues.

3.2 Aims

This second aspect to the study applies IRT in monitoring hibernation; investigating whether maintaining a skin temperature consistently above or below ambient temperature is linked to weight loss throughout the hibernation period. Further correlations aim to examine effects of pre-hibernation weight, length of hibernation and the frequency of spontaneous arousals, with comparisons between data from two winters. Outcomes will inform rescue centre protocols, perhaps providing a new monitoring method to ensure weighing and handling of individuals during hibernation is kept to a minimum and occurrences of artificially induced arousals reduced allowing a most natural hibernation period.

3.3 Methodology

This aspect of the study was conducted over the 2015/2016 winter and the 2016/2017 winter on *E. europaeus* undergoing rehabilitation at Prickles and Paws Hedgehog Rescue Centre, Cornwall, UK. The rescue centres protocols were incorporated into the project so no additional handling or disturbance was caused, which may have resulted in artificially induced arousal.

3.3.1 Housing

Individuals were housed in near-identical conditions; food, nesting material (shredded newspaper), lighting and temperature were consistent for individuals within each environment, shed (indoor hutches) and outdoors, with only hutch size showing slight variation within and between each environment (floor area ranged between $0.31m^2$ and $0.49m^2$). Differences in ambient temperature between the shed and outdoor area are accounted for in the analysis and this is only the case for the winter of 2015/2016 as for 2016/2017 all individuals sampled were contained in one large outside hutch system.

3.3.2 Hibernation assessment

Between November 2015 and March 2016, 21 individuals were monitored for spontaneous arousals, weight change and skin temperatures. Between December 2016 and March 2017, 14 individuals were monitored for spontaneous arousal only. Infrared images were taken (refer to section 2.3.1) within the nest box of hibernating individuals on the same day each week, throughout the 15/16 winter hibernation period, between 19.00 and 21.00. The FLIR E60bx was used throughout this section of the study. Bare skin (human) also has an emissivity of 0.98 (McCafferty, 2007) and therefore was also used for *E. europaeus* skin. Images were taken between 0.2 and 0.4m from the subject as determined by focus and clarity of the study area, this distance was set on the camera as <1m. A small hole was made in the nest material until a portion of the individual became visible (minimum area $3cm^2$), the individual was not touched and all material removed was replaced. The area analysed was the skin visible through the prickles, not the actual prickles.

Entry date into hibernation was defined as the first night that the individual did not leave the nest area, this was logged along with the last pre-hibernation weight. Individuals were weighed on a regular basis prior to hibernation, timing and frequency depended upon centre staffing/volunteers and weather. If individuals were displaying signs of entering hibernation, such as reduced food intake and green faeces (Bullen, 2010), they would not be handled. If they had not entered hibernation within the next three days they would then be weighed again. As a result, the length of time between last recorded weight and the start of hibernation varied between one and six days. Once in hibernation individuals would be weighed after approximately 6 weeks by centre staff to assess health and weight. For analysis *E. europaeus* were divided into two categories based on their weight prior to hibernation; either being 500-699g or ≥700g. This division was somewhat arbitrary, but based on historical data from the centre and staff experience which indicated that adult *E. europaeus* were equally frequent in these categories.

The date of final arousal from hibernation was defined as the first day of five consecutive days of activity outside the nest area. The timing of the post hibernation weight varied as staff induced arousal of some individuals due to weight loss (falling below or close to 500g). The maximum number of days a post-hibernation weight was recorded after the date of arousal from hibernation was six; this was due to staff wanting to ensure arousal from hibernation rather than consecutive spontaneous arousals and weather conditions preventing weighing of those in outside hutches.

3.3.3 Terminology

For the purposes of this study the following terms have been defined:

- Total hibernation period: The total number of nights between first entry into hibernation and final arousal, including nights with spontaneous arousals.
- Percentage spontaneous arousal: the amount of spontaneous arousal calculated as a percentage of the total hibernation period. Periods of spontaneous arousal began when an individual left the nest area. .
- Nights of hibernation: Hibernation period minus the number of nights with spontaneous arousal.

3.3.4 Statistical analysis

Weight losses (%) are presented as positive values, negative values on graphs refer to weight gain. Spontaneous arousal was converted to a percentage of the entire hibernation period to account for the different lengths of hibernation, thereby allowing fair comparisons. Spearman's rank correlations were carried out to establish significance of associations between weight loss and hibernation length or percentage of spontaneous arousal. Skin temperature was represented graphically as the mean difference between skin temperature reading and ambient temperature; allowing comparison between individuals regardless of varying ambient temperatures throughout the hibernation period. All statistical analysis was carried out in Minitab 17.

3.4 Results

3.4.1 Hibernation patterns

The maximum length of hibernation observed in the 2015/16 winter was 111 nights (106 hibernating, 5 nights spontaneously aroused) and 70 nights (68 hibernating, 2 spontaneously aroused) in the 2016/17 winter. The minimum length recorded was 13 nights and 5 nights for the 2015/16 and 2016/17 winters respectively. There was a smaller sample size for the 2016/17 winter, due to fewer individuals at the centre being eligible to hibernate. The mean hibernation length was similar for both winters 2015/16: 51.5 nights (SE.$\pm$7.5), 2016/17 winter: 48.8 nights (SE.$\pm$5.6).

For the winter of 2015/16 there was a significant positive correlation between weight loss and the length of hibernation (Figure 7, n=21 r_s=0.671, p<0.001), suggesting that weight loss is greater the longer the hibernation period. Weight data was not recorded for the 2016/17 winter. This result remained significant at the p<0.05 level when those individuals which gained weight during hibernation (due to the high number of spontaneous arousals in which they consumed food) are removed from the analysis (n=17, r_s=0.694, p<0.002). When divided into the two pre-hibernation weight categories of 500-699g (n=11) and $\geq$700g (n=10) the positive correlation remained statistically significant for each (Figure 10, 500-699g: r_s=0.882, p<0.001; $\geq$700g: r_s=0.802, p<0.005) demonstrating that weight loss increased with length of hibernation period regardless of pre-hibernation weight. Percentage weight loss however, was greater in the 500-699g category and all lost weight, whilst four individuals from the $\geq$700g category gained weight. When weight loss was plotted against total number of nights hibernating rather than total hibernation period (which includes spontaneous arousals), correlations remained significant (500-699g: r_s=0.855, p<0. 001; $\geq$700g: r_s=0.830, p<0.003).

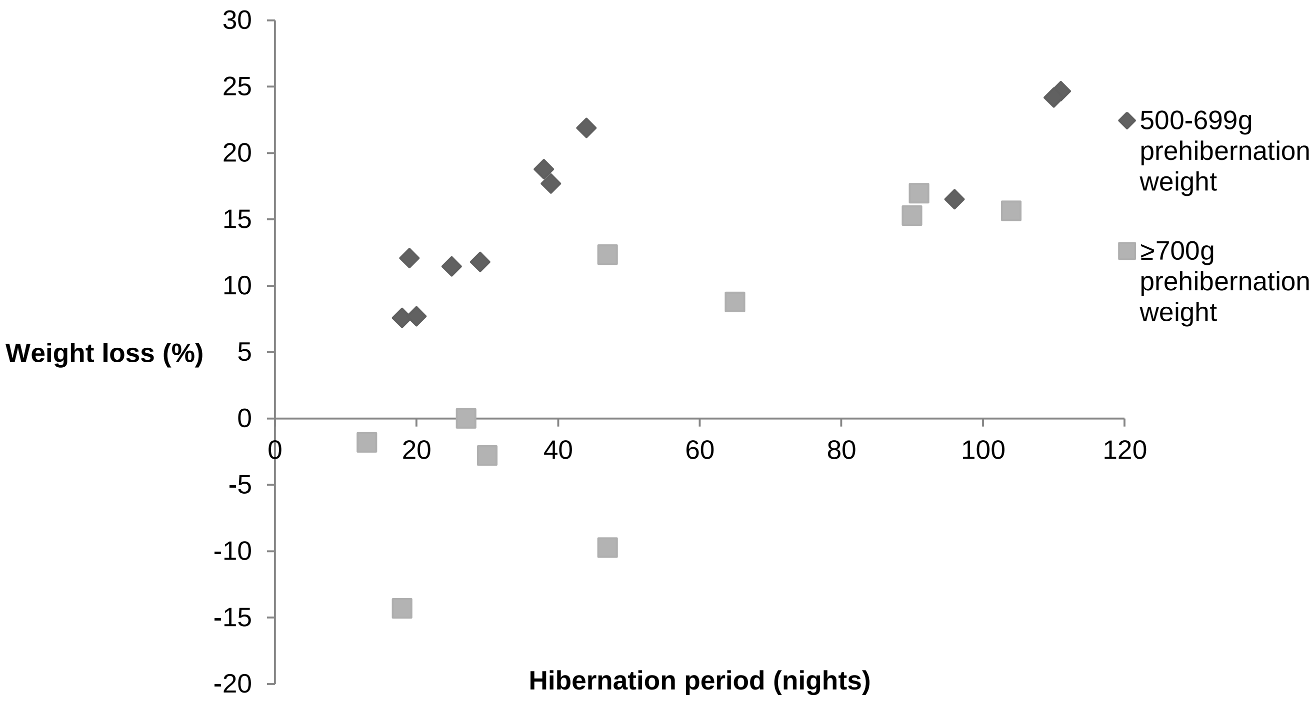


Figure 10: *E. europaeus* Weight loss and hibernation period (n=21, r_s=0.671, p<0.001), divided into the two weight categories 500-699g (n=11) and $\geq$700g (n=10), for the winter of 2015/2016 (500-699g: r_s=0.882, p<0.001; $\geq$700g: r_s=0.802, p<0.005).

Spontaneous arousals were observed in all individuals sampled bar one, who hibernated for only five nights. Food consumption during arousal was not recorded, however specialist small carnivore dried biscuit food was available throughout the hibernation period and weight gain demonstrated by four individuals shows that *E. europaeus* will eat during arousals. The maximum number of spontaneous arousals observed for a single individual in 2015/16 was 15 (total hibernation period 96 nights, including these arousals which accounted for 25 nights). In the 2016/17 winter three individuals displayed six arousals (total hibernation period for these three ranged between 41 and 66 nights, spontaneous arousals accounted for between 14 and 24 nights). Some spontaneous arousals lasted multiple nights, the longest being nine nights but the mean for the 15/16 winter was 1.6 nights (SE.$\pm$0.2) and 2.1 nights (SE.$\pm$0.3) for the 16/17 winter.

The mean number of nights between spontaneous arousals, for 2015/16 was 8.18 (SE.$\pm$1.75), with a calculated range of spontaneous arousals occurring between 2.5 to 36.6 days, which allows comparisons to be made to historic data sets. In 2016/17 there was a longer average of 9.9 days (SE.$\pm$1.9), but a smaller range of 3.2 to 22.6 nights.

There was a significant negative correlation between weight loss and percentage of spontaneous arousal during the 2015/16 hibernation period (Figure 11, n=21, r_s= -0.635, p<0.002). Individuals who lost most weight demonstrated fewer spontaneous arousals. When those which gained weight were removed from the analysis the result remained significant (n=17, r_s= -0.571, p<0.017).

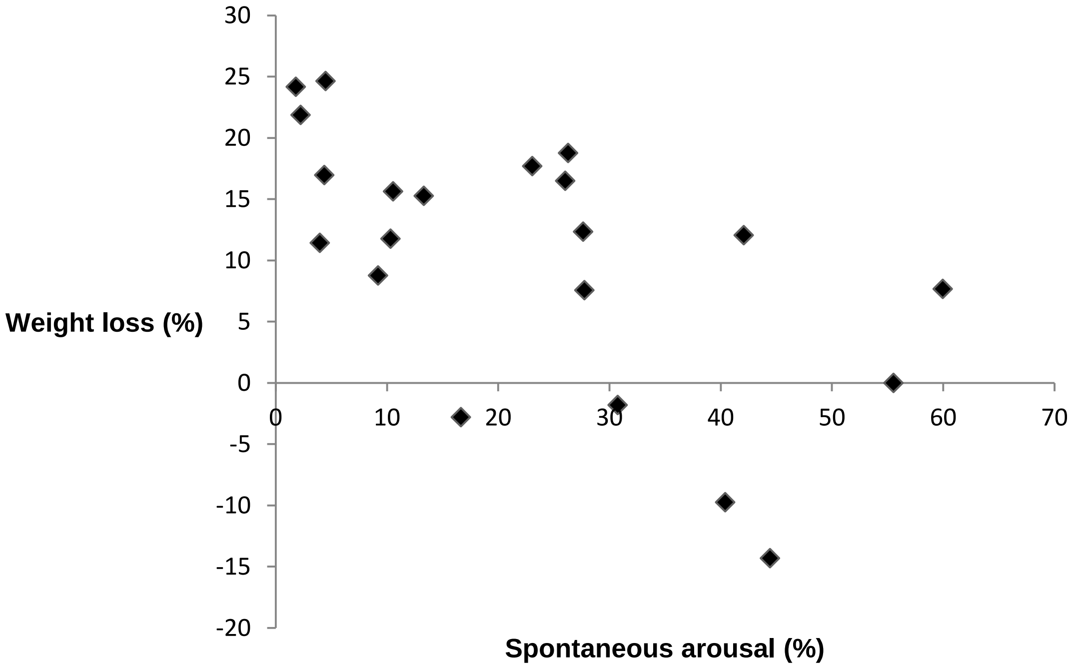


Figure 11: *E. europaeus* weight loss and spontaneous arousal, as a percentage of total hibernation period, for the winter 2015/2016 (n=21, r_s= -0.635 p<0.002).

Although length of hibernation varied between the two winters the rate of spontaneous arousal within each winter was further analysed. Correlations between spontaneous arousals and numbers of nights asleep were run for both winters' data, regardless of weight (Figure 12). There was a significant negative correlation when data from the 15/16 and 16/17 winter were combined (n=35, r_s= -0.605, p<0.001). Data from winter 15/16 were significantly correlated (n=21, r_s=-0.739, p<0.001), but those from winter 16/17 were not (n=14, r_s= -0.462, p=0.096). The latter winter's data was clustered between 45% and 65% spontaneous arousal and 5 to 25 days of hibernation. Hibernation length for the 2016/17 winter was noticeably shorter than the previous year, although the sample size was smaller.

As data on weight was only available for the 15/16 winter this was split into the two weight categories (Figure 13) 500-600g (n=11, r_s= -0.736, p<0.01) and ≥700g (n=10, r_s= -0.830, p<0.003) the result remained significant at the p<0.05 level, suggesting that pre-hibernation weight did not influence the pattern where individuals with a longer hibernation period had fewer occurrences of spontaneous arousal. This deviates from the expected higher spontaneous arousal rate for longer hibernation periods to reduce physiological risks such as oxidative stress and neuronal damage (Humphries *et al.*, 2003).

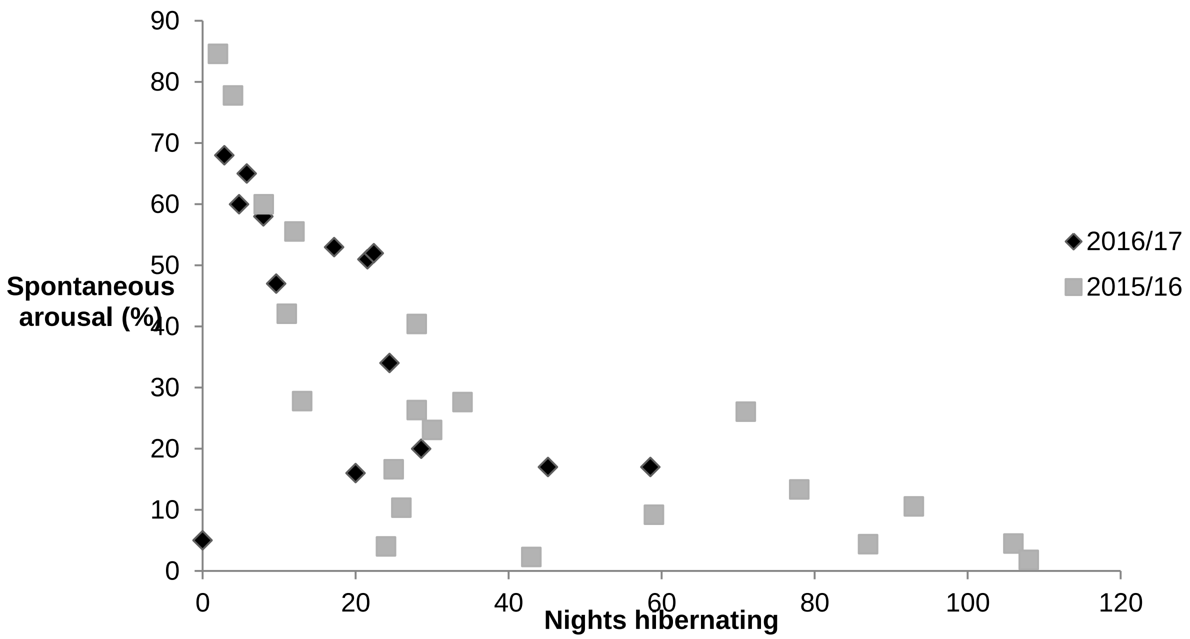


Figure 12: *E. europaeus* spontaneous arousal and nights hibernating for the winters 2015/2016 (n=21) and 2016/17 (=14).

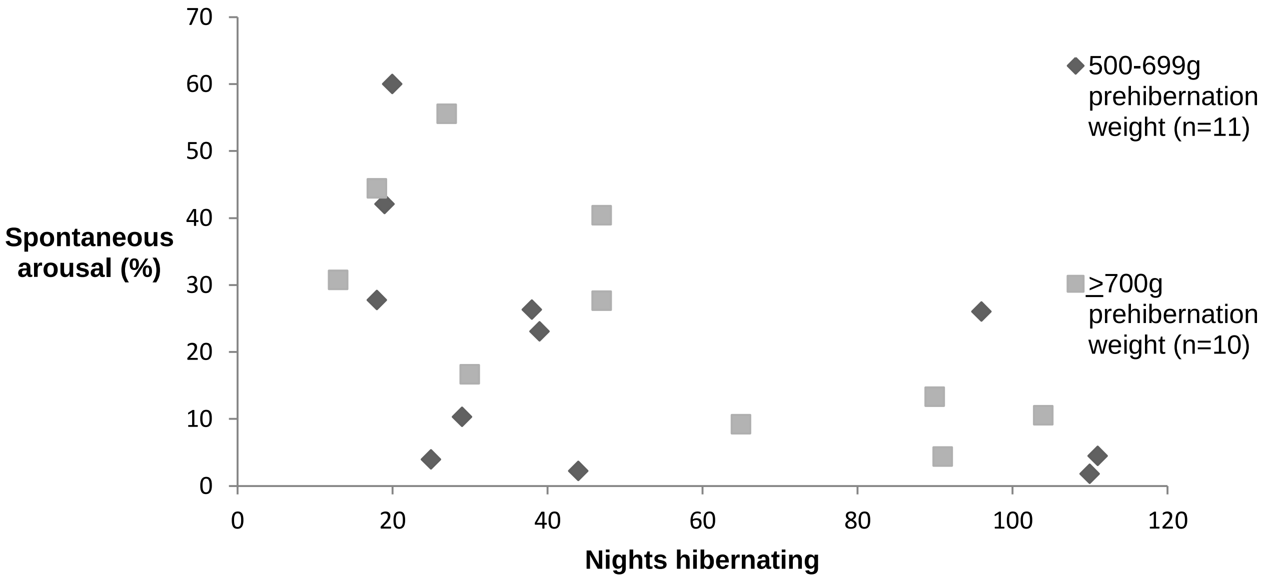


Figure 73: *E. europaeus* spontaneous arousal and nights hibernating within the two weight categories 500-699g (n=11, r_s= -0.736, p<0.010) and ≥700g (n=10, r_s= -0.830, p<0.003) during the winter of 2015/2016.

3.4.2 IRT hibernation monitoring

Skin temperatures were collected from within the nests of 17 individuals during the 2015/16 winter, the number of data points collected for each individual varied from two to 14, as some individuals displayed very short hibernation periods or were spontaneously arousing during the evening sampling. Ambient temperatures (not nest temperatures) on the days of sampling ranged from 14.8°C to 5.2°C. The mean difference of skin temperature and ambient temperature during hibernation for the winter of 2015/16 was plotted against weight loss for the 17 individuals (Figure 14). Each data point was labelled with the number of samples contributing to the mean value shown, it had no relation to weight loss which was calculated using pre and post-hibernation weights. There was no significant correlation, however only three of the 17 individuals had mean skin temperatures above ambient; the rest were below ambient.

Those individuals with a greater number of data points, indicating a longer hibernation are clustered around 15 to 25% weight loss and all displayed skin temperatures below ambient. This was also true for several individuals with only two or four data points but these appeared to be around the 15% weight loss mark and below. Those three individuals which displayed skin temperatures above ambient temperature (two within the lower weight category, one in the higher weight category) hibernated between 39 and 47 days, but with different numbers (1 to 19) of spontaneous arousals within this period.

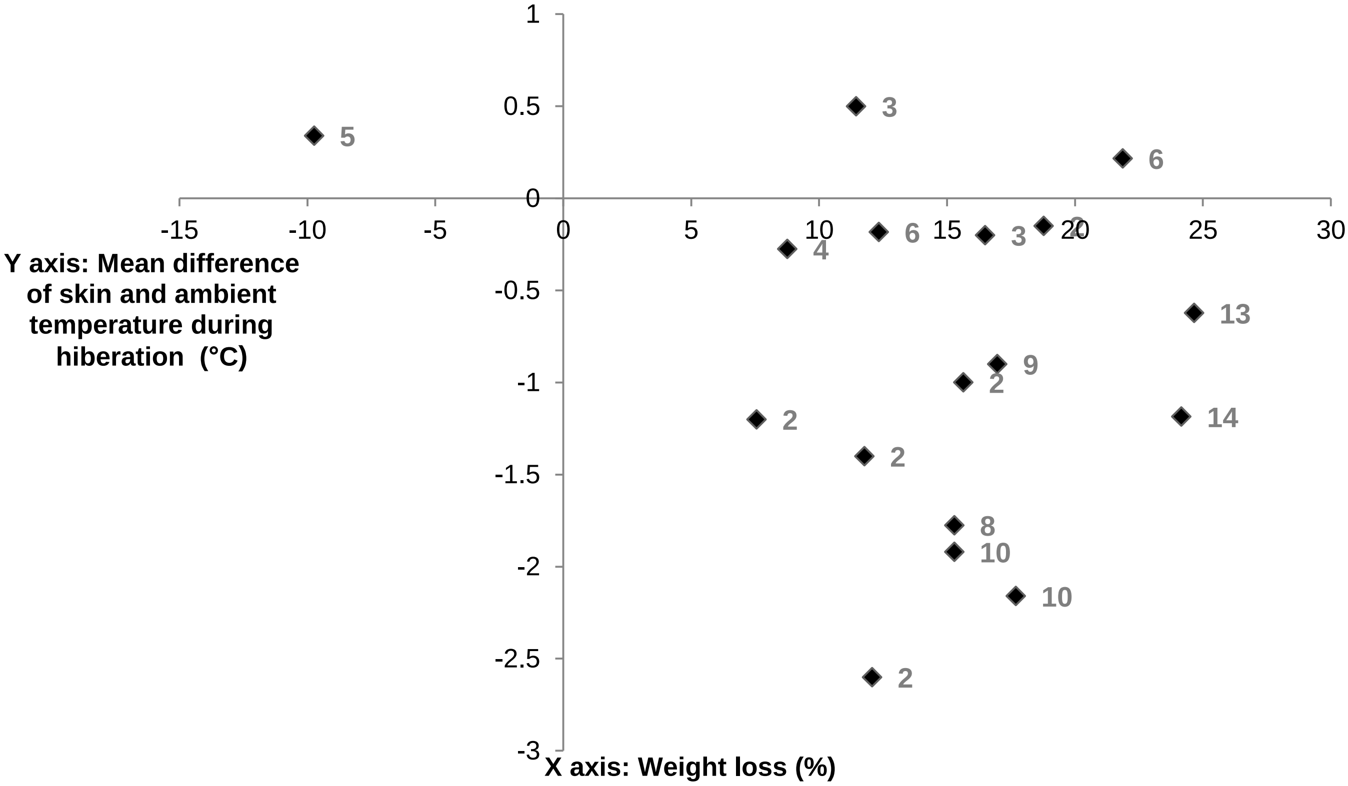


Figure 14: Mean difference of skin and ambient temperature throughout hibernation during the winter of 2015/2016 (y axis) and weight loss (x axis). Data labels represent the number of data points used to calculate the mean difference between skin and ambient temperature (n=17). A negative figure for weight loss represents a weight gain.

3.5 Discussion

The application of IRT to monitoring hibernation, although limited, provides insight into hibernation skin temperatures. The results indicated that most individuals sampled maintained a temperature below ambient, which is unexpected. Due to the methodology of ambient temperature being recorded within the nest box not within the centre of the nest and new application of infrared technology there is little confidence in these results so no assumptions of physiology can be made. Data collection proved difficult and even if results are not physiologically accurate, they are observable and consistent; however there was no clear pattern with weight loss which could currently influence or affect rehabilitation protocols.. The remaining hibernation data however, demonstrated significant correlations indicating higher weight loss for longer hibernation periods, and comparatively lower weight loss for individuals with a pre-hibernation weight of ≥700g. The unexpected result was fewer occurrences of spontaneous arousal observed in those individuals which demonstrated longer overall hibernation periods, a pattern which remained unaffected by pre-hibernation weight. The expectation would be increased occurrence of spontaneous arousals during a longer hibernation period as their purpose is believed to be prevention of oxidative stress, reduced immunocompetence and neuronal tissue damage (Humphries *et al.*, 2003). A longer hibernation would likely increase the risk of developing these complications/conditions. It could however be considered that those displaying high levels of spontaneous arousal did not enter a 'normal' or deep hibernation due to factors associated with being in a rescue (presence of food, different temperatures, hibernacula size and material). In this study spontaneous arousal was measured by leaving the nest, yet other research suggests spontaneous arousal can occur without leaving the nest (Walhovd, 1979; Reeve, 1994), so may have occurred in these longer periods of hibernation but went unrecorded. Spontaneous arousals did however negatively correlate with percentage weight loss, those displaying high levels of arousal losing less weight, perhaps suggesting that they ate during arousal as arousal itself has been previously suggested to be very energy expensive (Wang, 1978; Thomas and Geiser, 1997). These findings can be applied to rescue centres, resulting in reduced weighing and handling of individuals over 700g, once they have entered hibernation and demonstrates the need for further study into spontaneous arousal of *E. europaeus* within a rescue setting and the effect this has upon weight loss.

In comparison to its use in hypothermia assessment and diagnosis, IRT showed little value in the monitoring of hibernation within a rescue setting. Collecting multiple skin temperature images for the same individual, for the purposes of comparing skin temperature to ambient temperature and relating to pre and post hibernation weight as well as frequency of spontaneous arousals, proved difficult due to the frequency of arousals and short hibernation period of many sampled. The expectation was that weight loss would be linked to their skin temperature in relation to ambient temperature, those above ambient may be using more of their adipose reserves to do so. Statistically no significant relationship or correlation between percentage weight loss and mean difference between skin and ambient temperature was found, however only three of the 17 individuals sampled displayed mean skin temperatures above the ambient temperature contradicting the initial expectation. It could be assumed that these three individuals did not enter into a ‘full’ hibernation, however only one individual of the three gained weight and multiple individuals with shorter hibernation periods displayed temperatures below ambient. Despite similar housing and identical nesting material provided it cannot be ruled out that nest construction and therefore insulation may vary between the individuals. Any further study would require images to be taken more than once a week to ensure more data points per individual and further weight data to investigate the relationship between skin temperature, ambient temperature and weight loss. Observation of spontaneous arousal patterns during hibernation alongside pre and post hibernation weights however revealed a number of significant correlations and relationships (Figure 9 and 10).

The key finding which has applications for rescue centre protocols is that there was a statistically significant positive correlation between weight loss (%) and length of hibernation, demonstrating that weight loss increases with length of hibernation (Figure 7). This relationship was most significant for the pre-hibernation weight category of 500-699g, with these individuals losing a greater percentage of their body weight than those in the over 700g category. The implication for rescue centre protocols is to ensure greater monitoring of weight changes during hibernation of the 500-699g category. This could be through more frequent weighing despite the disturbance this may cause and monitoring of spontaneous arousals and consumption of food during these. Prickles and Paws current protocol is to induce artificial arousal from hibernation if weight falls below 500g, this is fairly standard across rescue centres and the only advice presently available. Very little data underpins this figure of 500g, (Reeve, 1994) and is an area for future research as it is so widely applied in rescue centres setting. Application of the pattern identified here however may allow rescue centres to be able to determine how regularly they weigh hibernating individuals by the frequency of spontaneous arousals (as well as taking into account pre-hibernation weight and length of hibernation).

Spontaneous arousals were observed in all individuals sampled bar one who hibernated for only five days. The mean number of arousals for each winter was very similar, falling within the ranges described in previous studies (Walhovd, 1979; Reeve, 1994 refers to Kristoffersson and Soivivo, 1964). The lower end of the range of number of days in between spontaneous arousals was similar to the range in Walhovd’s (1979) study, however the upper end of the range observed here is far greater than the 15 days described by Walhovd. In this non-invasive study, spontaneous arousal was only measured or recorded when an individual left their nest. This means that arousals which did not involve leaving the nest (as observed elsewhere: Walhovd, 1979; Reeve, 1994), could not be identified, leading to a greater range of days in between spontaneous arousals.

Many of the individuals sampled were kept in a warm environment until they reached a weight considered safe for hibernation, so the overall hibernation period of some will have been shorter due to environmental conditions (particularly ambient temperature but also day length) once in an outside hutch. Spontaneous arousals and weight loss were negatively correlated, with those demonstrating higher levels of spontaneous arousal, losing less weight or even gaining weight. This result was unexpected as literature suggests that spontaneous arousal is very energetically expensive (Wang, 1978; Thomas and Geiser, 1997). In rescue centres a small amount of specialist carnivore biscuits are available throughout hibernation, there is the opportunity for feeding to take place at every arousal. Although this was not recorded it could be a point for further investigation and may influence rescue centre protocols and frequency of weighing required. The data also indicates that some individuals aroused for multiple days at a time, the longest being nine consecutive nights, far greater than the 34-44 hours reported by Walhovd (1979) and up to 3 days reported by Reeve (1994) and Jensen (2004). Direct comparisons of mean length of spontaneous arousal with the present study cannot be made due to the different ways of measuring this variable. Multiple individuals exceeded these previous studies figures (Walhovd, 1979; Reeve, 1994; Jensen, 2004) which could indicate that many individuals are not entering deep hibernation perhaps due to the rescue environment and in particular presence of food as well as hibernacula and materials used being very different to those in the wild and probable greater level of disturbance. Being awake and out of the nest for nine consecutive nights may indicate arousal from hibernation rather than spontaneous arousal (typically <48 hours in this study). According to definitions used in this study, this individual had two hibernation periods in one winter. With such a small sample size it also raises the question whether this hibernation pattern/behaviour is found in other individuals and in the wild.

Establishing, with further data, the pattern of individuals' displaying high levels of spontaneous arousal losing less weight compared to others displaying lower levels of arousal, has the potential to influence rescue centre protocols. These individuals may require less weighing and handling, which reduces disturbance, allowing a more natural hibernation. This is important as handling or disturbance can artificially induce arousal, which has unknown effects upon weight loss (Pajunen, 1984; Reeve, 1994) and arousal has already been demonstrated to be very energetically expensive (Wang, 1978; Thomas and Geiser, 1997).

This study did not set out to examine the differences in hibernation patterns between the two winters; however it is worth noting that there are distinct differences. The sample size for the second winter was much smaller but this was in part due to less hibernating at the centre and only one outside hutch system being available for study as opposed to two the previous year. The key difference between the two years was length of hibernation, despite the mean hibernation lengths being similar the maximum hibernation length was much shorter for the 2016/17 winter, this was likely due to differences in environmental conditions between the two years. The average minimum temperatures recorded for February and March (2017) were warmer than the previous year (Newquay Weather Station, 2017), which may have also contributed to shorter hibernation periods as well as late entry into hibernation due to staff keeping individuals in a warm environment whilst administering treatments and waiting for sufficient weight gain before hibernation. The effects of climatic change on winter temperatures and weather could be an interesting point of study and comparison across both the UK and Europe on length of *E. europaeus* hibernation in future years. This could have implications for rehabilitation process based upon geographical location as well as rehabilitation success and therefore contributing to conservation of the species.

The long term effects of changes in hibernation pattern and duration (shorter or not at all) are not known. There is a clear change in hormone levels surrounding hibernation in *E. europaeus*, particularly in males where melatonin levels are elevated in autumn and decrease from January onwards whilst testosterone levels rise resulting in the males being fully sexually active upon arousal from hibernation (Reeve, 1994). The effects of a reduced or absent hibernation period on this seasonal activity and hormonal cycle are unknown and an area for further investigation.

Chapter 4: Summary

This project aimed to investigate the application of IRT for non-invasive hypothermia diagnosis in *E. europaeus*, using eye surface temperature as a proxy for core temperature, and skin temperature monitoring in hibernating individuals. The latter combined with observational data and patterns on spontaneous arousals and weight loss. With *E. europaeus* populations declining across the UK, improving diagnosis and husbandry practices with the application of new technology has the potential to improve success rates contributing to conservation of the species.

4.1 Hypothermia assessment

IRT, specifically the FLIR E60bx camera, can be used to accurately measure eye surface temperature, as a proxy for core temperature and therefore diagnose hypothermia non-invasively for *E. europaeus*. This non-invasive aspect is crucial to reducing handling, stress and shock, applicable to all wildlife but particularly for the species *E. europaeus* as they are difficult to uncurl and a rectal temperature cannot be obtained. Further research is required to cement these findings and extend to other taxa. The recommendation from this study is that the FLIR E60bx is an appropriate and accurate camera to diagnose hypothermia in *E. europaeus,* however this is the most expensive of the three cameras tested (FLIR E60bx, FLIR C2 and FLIR ONE). Application of the other two cameras would not allow an initial core temperature to be diagnosed without first developing a correction protocol for each individual camera, something which was not possible with the readings obtained through the thermal calibration trial conducted in this study. These cameras may however still enhance current staff diagnosis and would certainly allow eye temperature, as a proxy for core temperature, to be monitored in response to rewarming treatments, through relative difference in the temperatures observed. As the technology advances and becomes more widely available it is becoming more affordable. In the future FLIR E60bx cameras or other IRT cameras of a similar calibre may well be affordable within a rescue centres budget and contribute to improving survival rates as well as welfare and contributing to conservation of this declining species. The next step is to apply the use of IRT in establishing core temperature and developing rewarming protocols for *E. europaeus* at rescue centres, using the technology to monitor the warming process and adjust and tailor treatment to the individual increasing survival rates.

4.2 Hibernation monitoring

This study was unable to establish a clear relationship between a weekly skin surface temperature reading during hibernation and weight loss which could influence rehabilitation protocols. The majority of individuals mean temperatures were below ambient, which will likely have implications for weight loss. There is scope for a larger study to investigate potential relationships between skin surface temperature in relation to ambient temperature and weight loss. The key finding with applications for rescue centres from the hibernation data, although not unexpected, is that the longer an individual hibernates the greater the weight loss and that those with a pre-hibernation weight of ≥700g lost a smaller percentage body weight than those under 700g. Pre-hibernation weight was not however a factor in the pattern of fewer occurrences of spontaneous arousal during longer hibernation periods. Ranges of spontaneous arousal frequency do not match previous literature at the higher range end, likely down to spontaneous arousal only being measured by leaving the nest in this study, however this is standard practice used by rescue centres. There is however scope for further study and comparison of hibernation in a rescue setting and in a wild setting as hibernacula material and size are controlled in a rescue and the level of disturbance likely greater. Less invasive studies may provide data and results which are applicable within a rescue setting and could influence their hibernation protocols for *E. europaeus* to minimise handling and stress, particularly as handling has been reported to induce arousal, which appears energy expensive and is still not fully understood.

4.3 Wider applications and future research

This study has identified multiple modifications and developments that could be applied to assessment and husbandry practices of *E. europaeus* undergoing rehabilitation. The findings of this study are not just limited in application to UK rescue centres. *E. europaeus* are found across Europe (Figure 1) and with further research there is the potential to extend hypothermia or temperature assessment to other mammalian wildlife admitted to rehabilitation centres. Eye surface temperature as a proxy for core temperature, evidenced here in *E. europaeus* and in previous literature with sheep and cattle (Rainwater-Lovett *et al.,* 2009; Pérez de Diego et al., 2013), should be transferable to all mammals. The non-invasive aspect of IRT makes it ideally suited to wildlife as handling and stress can be reduced, yet accurate temperature assessments made.

This project has also identified a number of areas for further research from the continued exploration and application of different infrared camera models to further research into *E. europaeus* hibernation patterns in captivity. Further research into the energetics of hibernation, specifically the relationship between spontaneous arousals and weight loss has the potential to allow less invasive monitoring of hibernating individuals within a rescue centre setting, This also raises the question of what is a ‘safe’ hibernation weight and calls for further evidence to support the figures widely applied in rescue centres as the data sets these figures are based on are small. The patterns and variation observed here in terms of length of hibernation and frequency of spontaneous arousal within the two separate winters, as well as the fact that many individuals at the rescue did not hibernate at all raises the question of how *E. europaeus'* seasonal, hormonal and breeding cycle are affected by a reduced or no hibernation period and the role of rescue centres in this.

Although in this study the use of IRT to monitor skin temperature showed no clear relationship with weight loss, the skin temperatures on average were below ambient temperature. Physiologically this should not be the case; therefore repetition of the study with a reviewed methodology could uncover a clearer relationship of ambient and skin temperature. The key area of future research identified in this project is the development of hypothermia treatment/rewarming protocols that are based upon the core temperature initially diagnosed using the FLIR E60bx camera model as evidenced here. The use of FLIR E60bx to more closely monitor individual core temperatures would allow the implementation of rewarming protocols applied in medicine, small incremental temperature rises hourly or two hourly based upon an initial core temperature could be applied and the effects and response monitored non-invasively, with the FLIR E60bx. This has the potential to improve both welfare and survival rates.

Prickles and Paws Hedgehog Rescue aims to build on the findings of this study and continue developing husbandry and diagnostic protocols with the goal of increasing rehabilitation success rates.

Chapter 5: Appendices

Appendix 1: List of rescues and wildlife hospitals, number of *E. europaeus* admitted and if this number is and increase or decrease from 2015 (Wild Hedgehog Rehabilitators Forum 2017, Pers. comm., 27 February 2017).

Rescue Name	**No. Admitted 2016**	**Compared to 2015**
Prickles and Paws Hedgehog Rescue Cornwall	472	Increase
Hayley's Hedgehog Rescue Dorset	163	Increase
Brockworth Hedgehog Rescue Gloucestershire	302	Increase
Oxton Wild Hedgehog Rehab	162	Increase
Hedgehog Helpline South-east Wales	Approx 1000	Increase
Hedgehog Bottom	722	Increase
Hedgelina's Home for Hogs	146	Increase
South Essex Wildlife Hospital	Approx 1000	Not provided
Forth Hedgehog Hospital Scotland	185	Increase
Jersey Hedgehog Preservation Group	580	Increase
Selby Hedgehog Haven	48	Increase
East Sussex WRAS	480	Increase

Appendix 2: List of most common parasitic species of *E. europaeus* (Thamm *et al.,* 2009; Gaglio *et al.,* 2010; Whiting, 2012).

Endoparasites:

Nematodes:

- *Crenosoma striatum*
- *Eucoleus aerophilus*
- *3 Capillaria spp.* (*C. erinacei* , *C. ovoreticulata, C. aerophila*)

Trematodes:

- *Brachylaemus erinacei*

Protozoan oocysts

- *Isospora erinacei*
- *Isospora rategaievae*.

Ectoparasites:

- *Ixodes ricinus*
- *Ixodes hexagonus*
- *Archaepsylla erinacei*
- *Trichophyton erinacei*

Appendix 3: FLIR E60bx data sheet (FLIR, 2017a). Cost: £5,000 (M Clavey 2017, Pers. comm., 27 October).

FLIR E-Series bx

The New E40bx, E50bx and E60bx, Now with MSX® Advanced Thermal Imaging Camera Performance

Get more IR inspections done, create professional detailed reports easier, and share images and findings faster with FLIR's latest E-Series bx thermal imagers. Featuring a fresh array of imaging, communication, and productivity tools to help you take care of more customers.

FLIR Tools Mobile Communication – *Connect an E-Series bx camera to smartphones and tablets with our Wi-Fi app. Stream live video so customers can watch along. Import radiometric JPEGs, adjust levels and color, add measurement tools, then send images off in simple reports via email to get a faster yes for repairs.*

Superior Thermal Imaging – *Up to 76,800 pixels (320 × 240) for better long-range accuracy and the highest level of point & shoot camera IR resolution.*

MSX Thermal Image Enhancement – *See structural features, numbers, and other key visual details not normally apparent in a regular thermal image with an all-in-one thermal picture that instantly orients you to right where heat issues are.*

Onboard Digital Camera – *3.1MP resolution provides clear visible light reference pictures; includes built-in LED lamp that doubles as a flashlight plus a laser pointer to mark locations.*

Large Touchscreen with Auto-Orientation – *The E-Series touchscreen provides an intuitive interface that takes full advantage of the entire 3.5" display for easy access to diagnostics tools – now with auto-orientation to keep measurement and other data overlays upright no matter how you hold the camera.*

Picture-in-Picture – *Overlay thermal images onto digital scenes for extra location documentation.*

Multiple Measurements – *Add up to 3 box areas and 3 moveable spots using the touchscreen to gather more detailed temperature information*

MeterLink® – *Measure more than temperature. Send diagnostics data by Bluetooth from compatible clamp and moisture meters directly to E-Series bx cameras to embed extra information into thermal images as you capture them that further supports findings.*

Interchangeable Lenses and Manual Focus – *Optional wide-angle and telephoto optics let you capture more of a scene in one shot or get in closer to measure smaller, distant targets. Full focus control help you dial in the sharpest clarity and accuracy.*

Greater Temperature Sensitivity – *See subtler temperature patterns and detail with <0.045°C sensitivity.*

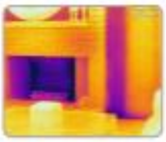
FLIR Tools Mobile WiFi Connectivity

Large 3.5" Touchscreen

Auto-Orientation Keeps Diagnostics Overlays Upright

Visible Light Pictures Align with Thermal Images: 3.1MP Digital Camera, LED Lamp, and Laser Pointer

Large Buttons for Easy Glove-Handed Operation



MSX Image Enhancement

Bluetooth® METERLINK® Communication

Imaging Specifications

FEATURES	FLIR E40bx	FLIR E50bx	FLIR E60bx
Thermal sensitivity (N.E.T.D)	<0.045°C at 30°C	<0.045°C at 30°C	<0.045°C at 30°C
Detector Type - Focal plane array; (FPA) uncooled microbolometer	160 x 120 pixels	240 x 180 pixels	320 x 240 pixels
MSX® Thermal Image Enhancement	Yes	Yes	Yes
Picture-in-Picture (P-i-P)	Fixed P-i-P	Scalable P-i-P	Scalable P-i-P
MPEG 4 Video Recording	Yes	Yes	Yes
Video Camera w/Lamp & Laser	3.1MP/LED Lamp/Laser Pointer	3.1MP/LED Lamp/Laser Pointer	3.1MP/LED Lamp/Laser Pointer
Digital Zoom	2X Continuous	4X Continuous	4X Continuous
Image modes	Thermal, Image Gallery, Visual	Thermal, Image Gallery, Visual	Thermal, Image Gallery, Visual
Image annotation	Voice (60s)/Text Comments	Voice (60s)/Text Comments	Voice (60s)/Text Comments
Moveable Spot	3 Spotmeters	3 Spotmeters	3 Spotmeters
Area Box	3 Area Boxes (full image with min/max/avg)	3 Area Boxes (full image with min/max/avg)	3 Area Boxes (full image with min/max/avg)
Delta T	Yes	Yes	Yes
Data Communication Interface	USB-mini, USB-A, Composite Video, Bluetooth, Wi-Fi	USB-mini, USB-A, Composite Video, Bluetooth, Wi-Fi	USB-mini, USB-A, Composite Video, Bluetooth, Wi-Fi
COMMON FEATURES			
Temperature range	-4 to 248°F (-20 to 120°C)		
Frame Rate	60Hz		
Field of view / Focus	25° x 19° / Manual (Minimum focus distance 1.3ft/0.4m)		
Spectral range	7.5 to 13μm		
Display	Built-in 3.5" color LCD		
Image Storage	>1000 radiometric JPEG images (SD card memory)		
Laser Classification/Type	Class 2/Semiconductor AlGaInP Diode Laser: 1mW/635nm (red)		
Set-up controls	Mode selector, color palettes, configure image info, units, language, date and time formats, and image gallery		
Measurement modes	Auto hot/cold spot, Isotherm (above/below/interval), insulation and humidity alarms		
Measurement Correction	Reflected ambient temperature & emissivity correction		
Battery Type/Operating Time	Li-Ion/ >4 hours, Display shows battery status		
Charging system	In camera AC adapter/2 bay charging system		
Shock/Vibration/Drop/ Encapsulation; Safety	25G, IEC 60068-2-29/ 2G, IEC 60068-2-6/ Drop-proof 2m (6.6ft) / IP54; EN/UL/CSA/PSE 60950-1		
Dimensions/Weight	9.7x3.8x7.2" (246x97x184mm)/<1.82lbs (825g), including battery		
Dimensions/Weight	9.7x3.8x7.2" (246x97x184mm)/<1.82lbs (825g), including battery		

Appendix 4: FLIR C2 data sheet (FLIR, 2017b). Cost: £550 (M Clavey 2017, Pers. comm., 27 October).

FLIR C2

Powerful, Compact Thermal Imaging System

The FLIR C2 is the world's first full-featured, pocket-sized thermal camera designed for building industry experts and contractors. Keep it on you so you're ready anytime to find hidden heat patterns that signal energy waste, structural defects, plumbing issues and more. The C2's must-have features include MSX® real time image enhancement, high sensitivity, a wide field of view, and fully radiometric imagery to clearly show where problems are and verify the completion of repairs.

Pocket Portable.

Keep it on you and at your side, ready for immediate use so you don't miss an opportunity

- Light, slim profile fits comfortably in any work pocket
- Brilliant 3" intuitive touch screen with auto orientation for easy viewing
- Built-in LED spotlight you can use as a flashlight and for photo illumination

Fully Radiometric.

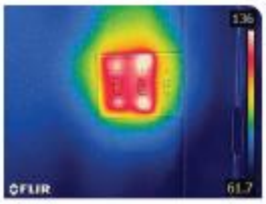
Save thermal image JPEGs instantly, then conveniently adjust and analyze them later with FLIR Tools to isolate temperature measurements on any pixel and create convincing reports

- MSX-enhanced thermal images provide stunning detail to help you identify problem areas easier
- Radiometric image stores 4800 pixels capable of capturing thermal measurements from -10°C to 150°C
- A wide FOV frames what pros need to see and high thermal sensitivity detects subtle temperature differences common in building applications

Easily Affordable.

Sub-$700 MSRP fits everyone's budget to help put this powerful tool into the hands of more people who can really use it

- FLIR Tools professional reporting software included – the industry standard in thermal image post analysis
- Streaming video via FLIR Tools, a feature not usually available on low-cost thermal camera systems
- FLIR's unique 2-10 warranty, covering parts and labor for two years and the detector for ten



Hot Overloaded Dimmer Switch

Warm Drain Pipe in Wall

Uninsulated Outside Wall

ww.flir.com/C2

FLIR | The World's Sixth Sense™

Imaging Specifications

Imaging and Optical Data	
IR sensor	80 × 60 (4,800 measurement pixels)
Thermal Sensitivity	<0.10°C
Field of view	41° × 31°
Minimum focus distance	Thermal: 0.15 m (0.49 ft.) MSX: 1.0 m (3.3 ft.)
Image frequency	9 Hz
Focus	Focus free
Spectral range	7.5–14 µm
3" Display (color)	320 × 240 pixels
Auto orientation	Yes
Touch screen	Yes, capacitive
Image presentation modes	
Thermal image	Yes
Visual image	Yes
MSX	Yes
Gallery	Yes
Measurement	
Object temperature range	–10°C to +150°C (14 to 302°F)
Accuracy	±2°C (±3.6°F) or 2%, whichever is greater, at 25°C (77°F) nominal
Measurement Analysis	
Spotmeter	On/off
Emissivity correction	Yes; matte/semi/glossy + user set
Measurments correction	Reflected apparent temperature Emissivity
Set-up	
Color palettes	Iron, Rainbow, Rainbow HC, Gray
Storage media	Internal memory stores at least 500 sets of images
Image file format	Standard JPEG, 14-bit measurement data included
Video Streaming	
Non-radiometric IR-video streaming	Yes
Visual video streaming	Yes
Digital Camera	
Digital camera	640 × 480 pixels
Digital camera, focus	Fixed focus
Additional Information	
USB, connector type	USB Micro-B: Data transfer to and from PC, iOS and Android
Battery	3.7 V Rechargeable Li-ion polymer battery
Battery operating time	2 h
Charging system	Charged inside the camera
Charging time	1.5 h
External power operation	AC adapter, 90–260 VAC input 5 V output to camera
Power management	Automatic shut-down
Operating temperature range	–10°C to +50°C (14 to 122°F)
Storage temperature range	–40°C to +70°C (–40 to 158°F)
Weight (incl. Battery)	0.13 kg (0.29 lb.)
Size (L × W × H)	125 × 80 × 24 mm (4.9 × 3.1 × 0.94 in.)
System Includes	
Infrared camera Battery (inside camera) Lanyard Power supply/charger with EU, UK, US, CN and Australian plugs Printed Getting Started Guide USB memory stick with documentation USB cable	

Appendix 5: FLIR ONE data sheet (FLIR, 2017c). Cost: £250 (M Clavey 2017, Pers. comm., 27 October).

FLIR ONE®

n FLIR ONE transforms your iOS or Android device into a powerful
advanced image processing includes FLIR MSX®, which blends
om the color camera with the thermal image to give you the best
e revolutionary OneFit™ adjustable-height connector ensures that
fit your phone or tablet, even when it's in a compatible protective
FLIR ONE app lets you stream thermal images to your smartwatch
and share images and videos with social media while keeping you
atest FLIR ONE tips and tricks. Explore the neighborhood, solve
ne house, and see the world from a new perspective with the new
ONE.

ERAS FOR BETTER IMAGING

orld with FLIR MSX® Blended
tter Image Detail and Clarity

camera and an HD 1440 x 1080 visible camera

al darkness to explore your world in a whole new way

hermometer gives real-time temperature of what you're looking at

NNECTOR

...se On - Adjustable Connector Means You Don't Have to Choose Between Thermal Vision and Safeguarding Your Device when Using Compatible Protective Cases

- Adjust length of USB-C and Lightening connector up to an additional 4 mm
- Reversible connectors for Android and iOS
- Secure the FLIR ONE to your mobile device while keeping your phone safe

POWERFUL APP

See Like a Pro – Real-Time Tips and Tricks, Connect to Your Smartwatch, Plus Easy Image Sharing

- Access tips and tricks to continually see new ways to use thermal imaging
- Stay connected to the FLIR ONE user community and easily share your images on social media
- See around corners and in awkward spaces by connecting to your Apple Watch or Android smartwatch

Specifications

General	FLIR One
Certifications	MFi (iOS version), RoHS, CE/FCC, CEC-BC, EN61233
Operating temperature	0 °C to 35 °C (32 °F to 95 °F) , battery charging 0 °C to 30 °C (32 °F to 86 °F)
Non-operating temperature	-20 °C to 60 °C (-4 °F to 140 °F)
Size	67mm W x34mm H x14mm D (2.6in x 1.3in x .6in)
Weight	34.5g
Mechanical shock	Drop from 1.5m (4.9ft)
Video	
Thermal and visual cameras with MSX	
Thermal sensor	Pixel size 17microns, 8 – 14microns spectral range
Thermal resolution	80x60
Visual resolution	1440x1080
HFOV / VFOV	50 ° ± 1 ° / 38 ° ± 1 °
Frame rate	8.7Hz
Focus	Fixed 15cm – Infinity
Radiometry	
Scene dynamic range	-20 °C to 120 °C (-4 °F to 248 °F)
Accuracy	±3 °C (5.4 °F) or ±5%, typical Percent of the difference between ambient and scene temperature. Applicable 60s after start-up when the unit is within 15 °C to 35 °C (59 °F to 95 °F) and the scene is within 5 °C to 120 °C (41 °F to 248 °F)
Thermal sensitivity (MRTD)	150 mK
Emissivity settings	Matte: 95%, Semi-Matte: 80%, Semi-Glossy: 60%, Glossy: 30 % Reflected background temperature is 22 °C (72 °F)
Shutter	Automatic/Manual
Power	
Battery life	Approximately 1h
Battery charge time	40 min
Interfaces	
Video	Male Lightning (iOS), Male USB-C (Android)
Charging	Female USB-C (5V/1A)
App	
Video and still image display/capture	Saved as 1440x1080
File formats	Still images – radiometric jpeg Video – MPEG-4 (file format MOV (iOS), MP4 (Android))
Capture modes	Video, Still image, Time lapse
Palettes	Gray (white hot), Hottest, Coldest, Iron, Rainbow, Contrast, Arctic, Lava and Wheel.
Spot meter	Off / °C / °F. Resolution 0.1 °C / 0.1 °F
Adjustable MSX distance	0.3m – Infinity
Battery charge monitor	0 – 100 %

Appendix 6: Criteria used for assessing newly admitted *E. europaeus* for hypothermia at Prickles and Paws Hedgehog Rescue (Prickles and Paws, 2017).

Questions to ask when assessing new arrivals for hypothermia:

- **Circumstance of rescue:** Was the hedgehog found out in the day? Was it 'sunbathing'? Was it washed out of a nest or found trapped in a pond, down a drain etc.? Was heat provided during transport to the centre?
- **Movement:** Does it appear drunk or wobbly? Are movements jerky? Does it seek heat / move on or off a heat pad if provided?
- **Reactions:** Is it struggling to curl tightly or not curling at all, lying 'flat'? Does it react to touch, sound, smell of food etc.? Are the prickles flat/ not raised?
- **Touch:** Is the abdomen cold to the touch? Can you see/feel the gums? Are they cold?
- **Eyes:** Are the eyes sunken and dull?

Almost all hedgehogs admitted will be suffering from some level of shock, a key symptom of which is hypothermia.

Diagnosis/ Category	**Symptoms / Signs**
Hyperthermic	Eyes bright, flat/not curling, may have infected wounds, rapid breathing
Normal	Tight curl, eyes bright, not wobbly, very responsive too sound or touch.
Mildly hypothermic	Can still curl, may be slightly wobbly or shaky, responsive to sound and touch, may be cool to the touch
Hypothermic	'Flat' or poor curl, unresponsive, cold to the touch, weak

With the above table consider the circumstance in which it was admitted to help make a diagnosis and inform heat provided and quantity and interval fluids are given at.

Information based upon veterinary advice, staff experience and Kay Bullen's Hedgehog Rehabilitation book (2010).

Appendix 7: Raw data of hypothermia diagnosis, based upon the E60bx being the most accurate, the category an individual has been placed in is determined by the E60 result and shows a comparison to the other two cameras.

Normal

E60	C2	ONE
35.2	35	36.1
35.5	34.5	37
35.2	34	35.7
35.5	33.1	33
35.4	33.6	36.1
34.8	33.4	34.8
34.4	33.1	36.8
35.9	34.1	36.7
35.1	34	31.9
34.3	33	34.9
35.3	34	37
34.7	33.1	35.6
35.5	32.6	29.3
34.9	33.7	36.4
34.2	33	29.8
35.5	34.4	32.1
34.9	32.8	35.5
35.4	34.6	36.2
35.7	34.8	35.8
35.7	34.7	37.1
34.4	34	29.3

Mild

E60	C2	ONE
33.9	32.9	32.6
32.1	30.8	35.2
33	32.1	34.1
32	30.9	33.1
32.3	31.2	32.1
33.6	33.7	36.1
33.3	31.4	32.1

Hypothermic

E60	C2	ONE
31.8	31.1	34.4
30.4	29.2	31.6
31.6	31.1	34
31.9	31	33.6
31.9	30.6	33.8
31.7	31	33.7
31.9	30.2	26.4
31.5	28.7	24.8
27.1	26	28.8
27.4	27.1	29.7
28.3	27.2	29.7
21.9	20.3	23.5
21.7	21.3	23.3
22.9	23.1	26.6
24.7	22.8	27.4
24.4	23.2	26.2
28.7	27.5	30.6
20.9	19.3	22.5
24.7	24	25.8
29.3	27.1	29.8
18.4	17.5	11.4
19.3	17.9	21.3
24.6	23.3	26.8
14.3	13.2	8.2
17	16.3	11.8

Highest reading of the three cameras

Lowest reading of the three cameras

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